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The Journal of Laboratory and Clinical Medicine

VOL. IV. ST. LOUIS, SEPTEMBER, 1919 NO. 12

ORIGINAL ARTICLES

AN EXPERIMENTAL STUDY UPON THE IMPREGNATION OF CLOTH WITH PEDICULICIDE SUBSTANCES*

BY W. MOORE, A.B., AND A. D. HIRSCHFELDER, B.S., M.D.,
MINNEAPOLIS, MINN.

THE problem of freeing the soldiers from lice divides itself into two distinct phases:

1. The laundering and bathing procedures for ridding the clothes and the men of lice or eggs already present. These were possible only behind the lines.

2. The use of sachet powders and impregnation of clothes and underwear with substances which would kill, or prevent the growth or even the presence of lice in the clothing. Since in many cases the soldiers were on duty continuously without change of clothing for three weeks or more, this phase of the problem assumed particular importance.

In spite of the very large number of researches, which reaches into the hundreds, that have been published or kept as confidential reports of the various governments since the beginning of the war, many difficulties have remained to be overcome, and it was to aid in this that the present series was undertaken at first under a research fund grant from the Regents of the University of Minnesota and later under the auspices of the Medical Science Division of the National Research Council. The researches upon the growing and breeding of lice, as well as upon conditions governing the laundering and ironing of clothing and the compounding of various sachet powders as well as a pre-

*From the Division of Economic Zoology and the Department of Pharmacology, University of Minnesota.

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Read before the Association of American Physicians, Atlantic City, June 18, 1919.

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liminary publication on the testing of a considerable number of substances used in the impregnation of cloth have already been published by one of us[1] and are not a part of the present paper. The entire study is now in press as a University of Minnesota monograph under our joint authorship and the detailed publication will soon appear.

The method at first employed in this research was to spread 1 gram of the substance to be tested upon a piece of cloth such as is used in the soldier's underwear 6×8 cm. in size, allow the substance to evaporate, and to test the duration of its power to kill lice by cutting off small strips at intervals and placing such a strip in a glass vessel in which a number of lice and eggs were kept. The vessel was then kept at incubator temperature and the percentage of the lice which were alive after 12-hour intervals was noted, as well as the ability of the various batches of eggs to hatch out.

In order to be regarded as effectual the substance being tested had to kill 80 per cent of the lice within 12 hours or 100 per cent in 24 hours.

All the lice were fed daily or twice daily upon the forearms of human subjects by Moore and a corps of assistants. That this was no indifferent procedure was shown by the fact that each of the persons doing so, suffered more or less from fever, rash, and glandular enlargement, and a condition somewhat resembling but not indentical with trench fever which has been described by Moore[2] in an article in the *Journal of the American Medical Association* and both of us in an article in the *Archives of Internal Medicine*. The testing of the substances upon the louse was all done by Moore,—the chemical syntheses by Hirschfelder.

The mere evaporation of the substances from the cloth exposed to the air was soon found not to coincide entirely with the disappearance of the substance when worn next to the skin and accordingly simple evaporation was discarded as a criterion and in the present research the substances were tested entirely by wearing a patch of impregnated cloth 6×8 cm. pinned to the undershirt and in contact with the skin of the wearer. These gave a fair indication of the conditions under which the substances would be used in practice and it was also possible to determine from the presence of reddening of the skin under the patch whether the compounds were too irritating to be used.

In the preliminary paper referred to above Moore has described the effects of impregnating the cloth with various lubricating oils, lard oil, fish oil, bone oil, and with various vegetable oils such as cottonseed oil, rapeseed oil and palm oil.

These were effective only in concentrations that would render the clothing so saturated with oil that they could not be worn and were only slightly effective when 1 c.c. to 8 square inches, enough to make the clothing visibly oily, were used.

Organic acids (salicylic, oleic, valeric) were useless. Thymoliodide failed to kill, phenyl iodide was transitory. Pure anthracene was useless but crude anthracene killed in 118 hours.

The metallic salts, copper oleate, zinc stearate, zinc chloride, ferrous sulphate, sodium arsenate in 2 per cent solution and saturated lead acetate gave negative results but saturated $HgCl_2$ killed.

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[1]Moore, W.: Jour. Am. Med. Assn., Aug. 17, 1918, lxxi, 530-531.
[2]Moore, W.: Jour. Am. Med. Assn., Nov. 2, 1918, lxxi, 1481-1482.
Hirschfelder, A. D., and Moore, W.: Arch. Int. Med., April, 1919, xxiii, 419-430.

Sulphur was negative. Iodoform which had been used effectively by the British in the N.C.I. (naphthalene 96%, creosote 2%, and iodoform, 2%) sachet powders was of some value, but the naphthalene killed only for 12 hours, though cloth impregnated with alphanaphthol retained its killing power for 360 hours and alphanaphthylamine for 720, after simple exposure to the air. On account of their toxicity the cloth was not worn, so that these figures are not comparable with the figures given when the cloth was worn.

Orthonitraniline which lasted 480 hours is too toxic to man to admit of its use. Heliotropine was found to have retained its killing power after exposure to the air for 528 hours, but on being worn lost it after 24 hours although if mixed with cocoa butter it lasted for 168 hours. This was due to the crystallizing.

The success of British investigators with naphthalene and creosote powders and our own results with cresol indicated that cresol was about the most useful substance, but that it disappeared from the clothing too rapidly to suit the purpose. It was accordingly suggested by one of us (H) that derivatives of cresol preserving the general cresol structure, but diminishing the volatility, might afford the solution of the problem, and on this account a number of cresol derivatives were synthesized.

After methylene di-cresol, cresyl benzoate and the cresyl phosphates and a mixture of di cresyl-ethers and cresyl ketone were found to be ineffective, a systematic study of the halogenated cresols was undertaken. Attempts made to prepare these by most of the methods described in Beilstein's Handbuch gave unsatisfactory results but satisfactory bromination could be secured by using Kopheschaar's mixture of sodium bromate, sodium bromide and hydrochloric acid.

Much better results were secured, however, by a method suggested by Dr. W. H. Hunter, dropping the calculated amount of bromine from a separating funnel directly into glacial acetic acid containing the cresol and a small piece of bright iron wire as a catalyzer. Bromination as shown by titrating a sample with KI and thiosulphate was complete in less than an hour. The mixture was then neutralized, separated, and filtered off, washed first with sodium thiosulphate to remove any excess of bromine or bromine that had entered the hydroxyl group, then with sodium bicarbonate and water and finally filtered or separated off.

Chlorine was introduced by bubbling into the glacial acetic acid solution of cresol in the presence of iron wire until the calculated weight of Cl had been taken up. The substances were otherwise treated in the same way as the bromine derivatives.

Iodine was introduced in saturated sodium bicarbonate solution in the presence of potassium iodide. A series of mono- and di-brominated chlorinated and iodo- ortho- meta- and para-cresols was thus prepared in which the halogen had entered the ring. By this mode of treatment the trihalogen compounds of the metacresol only are formed and no trihalogenated ortho and para compounds were obtained.

Chlorine was also introduced into monobrominated metacresol and in this way a monobrom, monochlor and a monobromdichlormetacresol was formed and in the same way a dibrommonochlormetacresol.

When patches of cloth impregnated with these substances were worn it was found that the monochlor orthocresol had no killing power after being worn 24 hours, and that the dichlor orthocresol killed 100 per cent after being worn 24 hours, but none after 48 hours.

The monoiodoorthocresol was variable and uncertain in its effect, but none of the samples killed more than 40 per cent of the lice after being worn more than 168 hours. The monobromparacresol and the monobrommetacresol lost their killing power after 48 hours, while the dibromorthocresol retained it for 120 hours and the dibrommetacresol for 240 hours. On the other hand, the tribrommetacresol possessed too low a volatility, and though lice were killed during the first 24 hours probably on account of the presence of a lower brominated impurity, after 36 hours the killing power was completely lost.

Evidently the optimum volatility to secure the persistence of louse killing power lay with the dibrominated cresol. An attempt was, therefore, made to prepare chlorbromcresols in order to see whether a still nearer approach to the optimum could be made.

The monochlormonobrommetacresol lost its killing power after 48 hours, and the monochlordibrommetacresol lost it after 48 hours, though it still killed 100 per cent of the lice that were exposed to its action for 36 hours. The dichlormonobrommetacresol, however, preserved its killing power for 312 hours (13 days), thus representing the best compound of the series. This compound which has not been described in literature is a white crystalline compound with a melting point of 62 degrees. When beechwood creosote was treated with two molar parts of bromine a product was formed which still killed after 240 hours. Tricresol treated with bromine naturally forms a mixture of bromocresols probably ranging from the mono- to the tribromcresol in varying amounts. When two molar parts of bromine were added and the mixture purified in the same way as the pure cresols the product possessed killing properties after having been worn for 96 hours. When three bromines were added it did so for 120 hours. A sodium salt of the dibrominated tricresol was prepared by (Moore) treating with an excess of 3 per cent NaOH. This compound possessed a high killing power for 192 hours and a low but persistent killing power for 360 hours.

We have also studied the aluminum, magnesium, and calcium compounds of cresol with a view to preparing insoluble compounds of the cresol derivatives which should be less alkaline than the sodium compounds and should slowly split off the cresol so as to continue its louse killing power. Though the compounds of magnesium and calcium with the halogenated cresols were not prepared it seems probable that these were even better suited than the sodium compounds for the purpose of the research. Since naphthaline was rated next to cresol in the experience of the British, corresponding chlorinated naphthaline derivatives were prepared by the method described by Emil Fischer, but these were less efficient than were the cresol derivatives.

After this series was completed the dibromxylenol, the monobromcarvacrol and the dibromeugenol were prepared by Professor R. A. Gortner.

Both the dibromxylenol and the monobromcarvacrol were much less volatile than the dibrommetacresol and killed just as rapidly. Dibromeugenol, which was

even less volatile than the tribrommetacresol, killed within 20 hours, while the tribrommetacresol failed to kill.

The effect of the monobromcarvacrol did not last any longer than that of the dibromcresol when worn, but the reasons for this were not determined.

The substances, therefore, which were found to be best suited to the purpose were the dibrommetacresol which lasted ten days and the dichlormonobrommetacresol which lasted 13 days; and these compounds seemed to fulfil the requisites of the problem so far as a laboratory trial could demonstrate.

Further pursuit of the problem was rendered unnecessary by the signing of the Armistice.

RELATION OF THE PANCREAS TO THE DIABETIC STATE*

By Dwight M. Ervin, A.B., Cincinnati, Ohio

THE pancreatic diabetic state is a condition characterized by several pathologic changes, absence of glycogen, hyperglycemia, fatty changes, acetone bodies and glucose in the urine; and a lowered respiratory quotient. This condition of diabetes has been ascribed to either of two changes from the normal; first the failure of the body cells to properly oxidize glucose; and second, the inability of the body to "fix" glycogen or a too rapid conversion of glycogen into glucose.

The failure on the part of the body cell to oxidize glucose has never been satisfactorily proved (Knowlton and Starling,[1] Levene and Mayer,[2] Paterson and Starling[3]). Levene and Mayer found that glucose disappeared more rapidly from a muscle preparation to which pancreatic extract had been added than from one in which no pancreatic enzyme had been placed. Yet the later work of Patterson and Starling[3] pointed rather to the fact that in the pancreatic extract preparation the large quantity of glucose that disappeared was converted into a nonreducing sugar, for if hydrolysis was carried on (1 per cent hydrochloric acid) the entire difference could be recovered.

The following experiments were performed to test the glucose consumption in the diabetic animal. The diabetic condition was produced by removal of the pancreas:

Experiment 1.—A well-fed dog of about 18 K. was used. Under ether the femoral artery and vein were exposed and a sample of blood taken from each, Sample 1 from the artery and Sample 2 from the vein. The leg was then sewed up and the pancreas removed. At the end of the sixth hour the dog was again placed under ether and the femoral artery and vein reopened. A sample of blood was again removed from each, Sample 3 from the artery and Sample 4 from the vein.

The glucose analysis of each sample gave the following:

Sample 1.—Femoral artery, before removal of pancreas	0.177%
Sample 2.—Femoral vein, before removal of pancreas	0.141%
Difference = normal glucose consumption of leg	0.036%

*From the Department of Pathology of the University of Cincinnati, Cincinnati, Ohio.

Sample 3.—Femoral artery, after removal of pancreas	0.260%
Sample 4.—Femoral vein, after removal of pancreas	0.225%
Difference = Diabetic consumption of glucose by leg	0.035%

Urine after removal of pancreas and at the time Sample 3 and 4 were taken glucose 1.67%.

During the passage of the blood through the leg in a normal condition the glucose concentration was reduced from 0.177% to 0.141% by tissue utilization. In the diabetic state the reduction was the same. In the normal it was reduced 0.036%, in the diabetic it was reduced 0.035%. The glucose consumption was not interfered with in any manner.

Experiment 2.—A rabbit (2 K.) well fed on carrots, greens, and lettuce was placed under ether and blood removed from femoral artery and vein, Sample 1 from the artery and Sample 2 from the vein. The pancreas was then removed. In one hour the rabbit had recovered from the anesthetic. The urine reduced Fehling's solution. At the end of the sixth hour the reduction was strong. Ether again was given and blood taken from the femoral artery and vein. Sample 3 from the artery and Sample 4 from vein.

The glucose analysis of each sample

Sample 1.—Femoral artery, before removal of pancreas	0.130%
Sample 2.—Femoral vein, before clamping vein of pancreas	0.160%
Difference = normal glucose consumption of leg	0.036%
Sample 3.—Femoral artery, after removal of pancreas	0.270%
Sample 4.—Femoral vein, after removal of pancreas	0.240%
Difference = diabetic consumption of glucose by leg	0.035%

Before removal of the pancreas the sugar of blood was reduced in its circulation through the leg by 0.036%. After depancreatization it was reduced 0.030%. A difference of 0.006% which is within the limits of error.

Experiment 3.—While under ether a dog's femoral artery and vein were exposed and the large pancreatic vein found. The smaller veins were tied off. A glucose solution (20 c.c. of 18%) was injected into the ileum. After ten minutes blood was taken from femoral artery and vein, Sample 1 from artery and Sample 2 from vein. The pancreatic vein was then clamped off gently and after an interval of ten minutes blood Sample 3 was taken from the femoral artery and blood Sample 4 from femoral vein. The vein of the pancreas was then unclamped and after another interval of ten minutes blood Sample 5 was taken from the femoral artery.

Analysis of the glucose in each sample

Sample 1.—Femoral artery, before clamping vein of pancreas	0.188%
Sample 2.—Femoral vein, before clamping vein of pancreas	0.160%
Difference = normal consumption of glucose by leg	0.028%
Sample 3.—Femoral artery, after clamping vein of pancreas	0.236%
Sample 4.—Femoral vein, after clamping vein of pancreas	0.208%
Difference = consumption of glucose by leg in diabetic state	0.028%
Sample 5.—Femoral artery after unclamping pancreatic vein	0.190%

From Sample 5 we have a check upon the effect of ether. The rise in arterial blood sugar from 0.188% to 0.236% was due to the clamping of the pancreatic vein. Here again although we have a decided rise in the percentage of sugar in the blood on the clamping of the vein of the pancreas, we have the same reduction in the arterial sugar on its flow through the leg. If in the condition of diabetes there is a underoxidation of glucose the percentage of glucose in the blood should remain the same after it flows through the leg. But in the three preceding experiments the decrease in the percentage showing the utilization by the tissues, was the same in the deprancreatized state as in the normal. This, however, is only claimed within the limits of a period of six hours after depancreatization. Further experiments upon the effect of time will be undertaken.

It may be argued that six hours is not sufficient time to allow for an interference of oxygen to take place, and that while the pancreas may have been removed, its internal secretion was still present in the blood controlling the oxidation of glucose. If such be argued we must then admit that hyperglycemia and glycosuria are phases of the diabetic state independently of oxidation in the tissues. In the above experiments a hyperglycemia and glycosuria were produced by the removal of the pancreas, while the oxidation remained normal.

The hyperglycemia of the diabetic state receives no satisfactory explanation upon the theory of underoxidation unless a full storage of glycogen is present. In the body glucose is rapidly converted into glycogen until finally there is reached a condition of equilibrium represented by the equation:

$$\text{Glucose} \rightleftarrows \text{Glycogen}$$

In such an equilibrium should the gluose increase, the quantity of glycogen must also increase. But in the diabetic state there is little or no glycogen present. This is the very opposite from that which we would be led to expect from the mass reaction of glucose to glycogen.

Without a full storage of glycogen through the tissues the underoxidation can not satisfactorily explain the hyperglycemia. Many observers have found that the diabetic liver does not store glycogen. Minkowski[4] observed that depancreatized dogs had no storage of glycogen in their livers. It has been confirmed by many others. Epstein and Baehr,[5] Claude Bernard,[6] Naunyn,[7] Lepine,[8] Demyer,[9] and Hedon.[10] Another explanation of the hyperglycemia with its glycosuria than that given by underoxidation is necessary.

The hyperglycemia is readily explained if we assume glycogenic function for the pancreas. This theory of the nature of the function of the internal secretion of the pancreas is supported embryologically as well as experimentally. In general it is believed that the "islands of Langerhans" are the gland cells of the internal secretion. Embryologically they arise from the intestinal tract associated with a gland which has an external secretion that hydrolyzes the starches, and remain associated with this gland. The development of the islands is intimately related to the development of the acini. It is logical to conceive them from their development and position as modification of the acini of this gland.

This is not a functional modification, but a structural one, in order that the secretion may be diverted into the portal blood to act as synthetic enzyme while the external passing out through the duct is a hydrolyzing enzyme. This hydrolysis or splitting of starches into glucose may be represented as follows:

$$\text{Starch} + \text{Enzyme} \rightleftarrows \text{Glucose} + \text{Enzyme}.$$

The splitting of the starch proceeds until there is a certain ratio of glucose to starch or until a certain equilibrium is reached. If, however, the glucose is removed from the field of reaction as in the body by diffusion through the wall of the intestinal tract, the entire amount of starch is hydrolyzed. The enzyme does not change the nature of a reaction, but only its velocity, which it increases.

If we now conceive that the internal secretion poured into the portal blood stream from the structurally modified islands has the same function as the external

secretion, we are able to follow the fate of the glucose after it reaches the portal circulation. As the glucose diffuses through the intestinal wall and into the portal blood it is met by the internal secretion. Since it is entirely glucose the reaction becomes synthetic and the enzyme gives the needed velocity. The reaction will be:

$$\text{Glucose} + \text{Enzyme} \rightleftarrows \text{Glycogen} + \text{Enzyme}.$$

As the glycogen passes through the liver it is removed from the blood. This may be represented diagrammatically (Fig. 1). Conversely if the internal secretion does not reach the portal system a slow and inefficient synthesis with consequent hyperglycemia and glycosuria will result. When it is realized that at most there is only three to five grams of glucose in the blood and a hundred grams will be absorbed in an hour the need of swift synthesis into glycogen is seen.

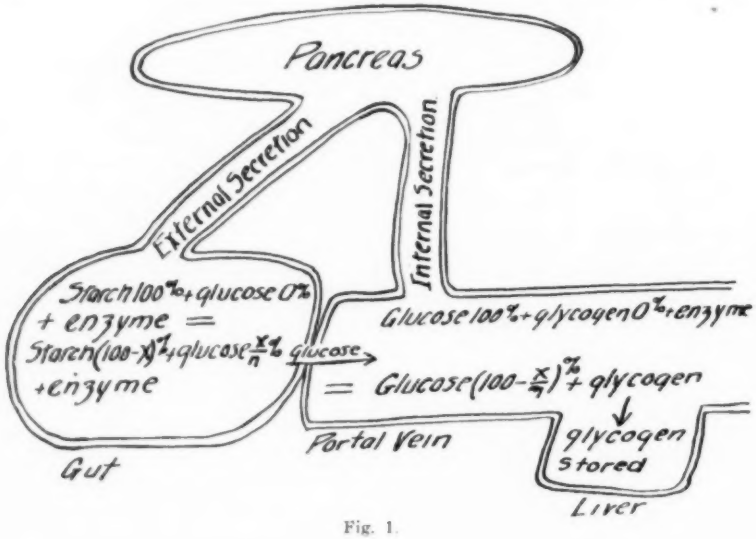

Fig. 1.

Here then, if we can prove the glycogenic function of the internal secretion of the pancreas, we find an explanation of the hyperglycemia and corresponding absence of glycogen. In addition I expect in subsequent articles to show that this absence of glycogen in itself is sufficient to explain the fatty changes, the acetone bodies, the disturbed respiratory quotient, and the failure of a carbohydrate diet to raise the respiratory quotient or check the acetonuria in the state of pancreatic diabetes.

ANIMAL EXPERIMENTS UPON THE GLYCOGENIC FUNCTION OF THE PANCREAS

Experiment 1.—A rabbit (2,000 grams), starved for eight days, was put under ether and its abdomen opened. Sample 1 of blood was taken from left side of heart with syringe. The pancreatic vein was then clamped and Sample 2 taken from left side of heart. The vein was then unclamped and an interval of ten minutes allowed. Glucose solution (20 c.c. of 18 per cent) was then injected into the ileum. In five minutes Sample 3 was taken from left side of heart. Again the vein was clamped and after five minutes Sample 4 was taken from left side of heart.

The Analysis of Samples

Sample 1.—Blood sugar, before clamping pancreatic vein	0.176%
Sample 2.—Blood sugar, after clamping pancreatic vein	0.175%
Sample 3.—Blood sugar, with glucose in gut	0.208%
Sample 4.—Blood sugar, pancreatic vein clamped with glucose in gut	0.308%

The clamping of the pancreatic vein prevented the removal of the glucose from the blood. The sugar concentration did not rise when there was no glucose in the gut and the vein of the pancreas was clamped. When the glucose was placed in the gut and the vein free the concentration rose 0.033%. But when the vein was clamped with the glucose in the gut the concentration in five minutes rose 0.100% about three times as rapidly. (Chart 1, Curve 1.)

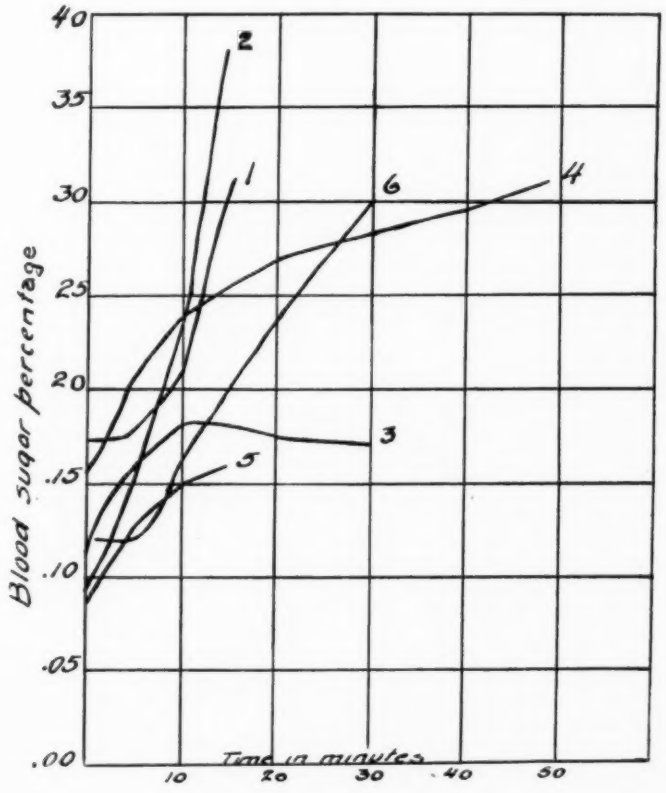

Chart 1.

Experiment 2.—A rabbit was put under ether and the abdomen opened. Blood Sample 1 was taken from the left side of heart. A glucose solution (20 c.c. of 18 per cent) was injected into the ileum. Ten minutes later Sample 2 was taken from left side of heart. The pancreas was then totally removed. After five minutes Sample 3 was taken from left side of heart. One c.c. of urine was found in the bladder and removed. It reduced Fehling's solution. The analysis gave the following (Chart 1, Curve 2):

Sample 1.—At beginning of experiment	0.094%
Sample 2.—After injection of glucose in gut	0.200%
Sample 3.—After removal of pancreas	0.386%

A hyperglycemia resulted.

Experiment 3.—A rabbit (1,900 grams) was put under ether. Blood samples were all taken from left side of heart. Sample 1 was taken five minutes after animal was under anesthetic. The remaining samples were taken as indicated below, after the injection of 19 c.c. of 10 per cent solution of glucose into ileum.

Sample 1.—At beginning	0.118%
Sample 2.—Five minutes after glucose was in gut	0.159%
Sample 3.—Ten minutes after glucose was in gut	0.174%
Sample 4.—Twenty minutes after glucose was in gut	0.168%
Sample 5.—Thirty minutes after glucose was in gut	0.165%

Experiment 3 gives the rise in the normal. An absorption of two grams per kilo in the normal rabbit gave a rise of sugar in the blood of only 0.056 per cent (Chart 1, Curve 3).

Experiment 4.—A rabbit (1,900 grams) was put under ether and the pancreas removed. Eighteen c.c. of 10 per cent glucose was injected into the ileum. Blood samples from left side of heart were taken as indicated below.

Sample 1.—Just before injection of glucose	0.165%
Sample 2.—Five minutes after injection of glucose	0.198%
Sample 3.—Ten minutes after injection of glucose	0.240%
Sample 4.—Twenty minutes after injection of glucose	0.265%
Sample 5.—Thirty minutes after injection of glucose	0.280%
Sample 6.—Forty minutes after injection of glucose	0.295%
Sample 7.—Fifty minutes after injection of glucose	0.305%

Urine at end of experiment reduced Fehling's solution. The total rise was 0.140 per cent. A hyperglycemia developed with its related glycosuria (Chart 1, Curve 4).

Experiment 5.—A rabbit (2,000 grams) was put under ether—20 c.c. of 10 per cent glucose injected into ileum. The blood samples from left side of heart were taken as given below (Chart 1, Curve 5):

Sample 1.—Immediately after injection of glucose	0.090%
Sample 2.—Five minutes after injection of glucose	0.130%
Sample 3.—Fifteen minutes after injection of glucose	0.150%

Again the rise in the non-depancreatized animal was insignificant.

Experiment 6.—Rabbit, weight 1,800 grams, was put under ether. All blood samples were taken from left side of heart at times indicated below. A solution of glucose was injected into ileum and the pancreas removed. Glucose analysis of blood.

Sample 1.—Before pancreas was removed	0.122%
Sample 2.—Five minutes after removal of pancreas	0.115%
Sample 3.—Ten minutes after removal of pancreas	0.156%
Sample 4.—Twenty minutes after removal of pancreas	0.237%
Sample 5.—Thirty minutes after removal of pancreas	0.300%

Here as in the other experiments when the pancreas is removed the rise of glucose is rapid and high.

In all of these animal experiments the rise of percentage of blood sugar when 2 grams of glucose per kilo was given through the intestinal tract varies widely according to whether the animal is normal or depancreatized.

In the above experiments we find two different conditions of the percentage of sugar in the blood. In the normal animal we find that 2 grams of glucose per kilo does not produce a severe rise in the percentage in the blood, but in the depancreatized animal the rise is quick and severe enough to bring about a glycosuria. In the normal animal the glucose diffusing into the portal blood is met by the internal secretion of the pancreas and rapidly converted into glycogen. The rate of conversion is such as to prevent a hyperglycemia of such proportion as

to produce a glycosuria. But in the depancreatized animal the conversion does not take place and a hyperglycemia and glycosuria results. The hyperglycemia is due to the lack of the internal secretion of the pancreas.

With this fact we are in a position to discuss the rate of absorption of glucose from the intestinal tract and the rate of its conversion into glycogen, to the rise in concentration of the blood sugar. It is this relation that is altered in the state of pancreatic diabetes. In the normal individual the rate of hydrolysis of starch and its absorption as glucose is below the rate of the conversion of the glucose into glycogen. When this is changed and the rate of conversion of the glu-

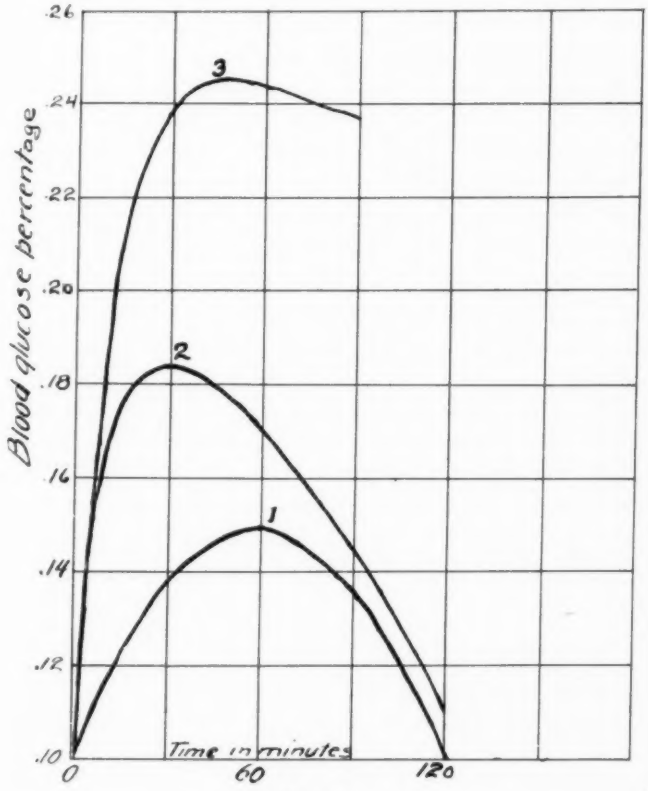

Chart 2.

cose to glycogen becomes less than the rate of the absorption of glucose from hydrolysis of starch, the blood sugar rises, there is a hyperglycemia, and glycosuria develops. The enzyme for the rapid conversion is the internal secretion of the pancreas. The rate of absorption of ingested glucose is different. An individual in normal condition will upon the ingestion of one hundred grams of glucose show a rise in the concentration of the blood sugar of 0.040 per cent to 0.050 per cent in an hour. At the end of the second hour the blood is again normal. Curve 1, Chart 2 shows the curve of a normal individual upon 100 grams of glucose. A hundred grams of glucose given per mouth does not produce a hyperglycemia high enough to give a glycosuria, because it is too rapidly converted into glycogen. In the diabetic state where the internal secretion of the pancreas is at fault the con-

version of glucose into glycogen is too slow and hyperglycemia of severity is the result. This slow conversion of glucose into glycogen is the state of diabetes.

Again in Chart 2, Curves 2 and 3 are the curves of a diabetic. Curve 3 gives the rise of the blood sugar upon feeding a hundred grams of glucose. In this case the blood sugar rose from 0.10 per cent to 0.240 per cent in one hour. At about 0.18 per cent glycosuria becomes noticeable. The rate of rise in the diabetic is very much greater than in the normal. Curve 2 is the same patient upon 30 grams of glucose. The rise is almost as rapid as shown in Curve 3 yet at the end of half an hour has reached its crest and at the end of two hours had returned to normal. The difference between these curves is not because of oxidation, but because of the change in the rate of conversion into glycogen and its storage. The condition represented by Curves 2 and 3 is exactly the same condition we found in the depancreatized animals. When the animal is depancreatized it is no longer able to convert the absorbed glucose into glycogen, no glycogen is stored and a hyperglycemia with glycosuria develops; the diabetic through a disease of the pancreas in a similar way loses the internal secretion, fails to convert glucose into glycogen—and to the degree that he lacks this internal secretion he develops a hyperglycemia and glycosuria. If we estimate the consumption of glucose per hour as 15 grams in a resting individual (360 grams per day) and feed this to a diabetic we should expect to see the curve of the blood sugar return to normal at the end of the hour. This is what happens as shown in Curve 2, Chart 2.

Not only in the depancreatized animal, but also in the diabetic person, the external secretion of the pancreas acts as a synthetic enzyme upon the absorbed glucose. When the pancreas is functioning, the person converts his glucose into glycogen at a rate sufficient to prevent a severe rise in his blood sugar; when it is not functioning, he is unable to convert the glucose at a sufficient rate and the percentage of glucose in the blood rises, giving a hyperglycemia and glycosuria.

SUMMARY

The experiments show that a depancreatized animal as long as six hours after depancreatization develops a hyperglycemia and glycosuria just as in the true state of pancreatic diabetes, yet consumes glucose at the same rate as the normal animal. The hyperglycemia and glycosuria are dependent upon the rate of synthesis of glucose into glycogen and not upon interference with the normal rate of oxidation.

We believe that the internal secretion of the pancreas is an enzyme, similar to the external, but diverted into the portal blood for the rapid synthesis of glucose into glycogen.

The failure of its action is the cause of the state of pancreatic diabetes; a diabetic is one who fails to synthetize the absorbed glucose into glycogen at a sufficiently rapid rate to prevent a hyperglycemia.

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STUDIES ON CHOLESTEROL*

VI. The Value of Blood Cholesterol Determinations and Their Place in Cancer Research

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THE cholesterol content of the blood has been studied thus far mainly for the purpose of scientific investigation, and its clinical significance has received but little attention. With the exception of Joslin, Rothschild, and perhaps a few others, clinicians seem to be under the impression that cholesterol determinations have little or no practical value.[7, 8]

The observations I was able to make between November, 1915, and November, 1918, strongly suggest, however, that the test for cholesterol is capable of giving valuable clinical information, although it should not be looked upon as a specific, diagnostic test, such, for example, as the Wassermann test. It might fitly be compared to the test for albumin in the urine, the clinical value of which is beyond dispute, even though the latter does not enable us to differentiate between cystitis and nephritis without other data.

Rothschild has pointed out that in cholelithiasis the absence of a constant hypercholesteremia is due chiefly to the rate of precipitation of the cholesterol in the gall bladder, hence low blood cholesterol values would naturally occur if the surplus cholesterol had been used up in the making of the gallstones, while high blood cholesterol values associated with gall bladder symptoms, in the absence of stones, might merely indicate that the gallstones had not had time to form; that continued hypercholesteremia is well calculated to lead to the formation of gallstones is self-evident.

We are still in the dark concerning the chemical factors which cause the bile-cholesterol to crystallize to gallstones around a nucleus of bacteria and desquamated cells in some cases, while in others no gallstones are formed, although both bacteria and cell detritus are present. Rosenow obtained cultures of pathogenic bacteria (streptococci, bacilli Welchi, colon, typhoid bacillus, etc.) from the core of ordinary mixed gallstones in 29 of 34 experiments, but found no pathogenic bacteria in 4 pure cholesterol stones. The latter are obviously formed by a more rapid type of precipitation, although we do not know the factors by which this precipitation is brought about. We know, on the other hand, that "typhoid-carriers" may harbor typhoid bacilli in their gall bladders for

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many years without being afflicted with cholelithiasis. Since the cholesterol content of the bile must be influenced by the cholesterol content of the blood, the study of the latter is eminently fitted to furnish data concerning the cholesterol metabolism resulting in the formation of gallstones.

Apart from the problems connected with cholelithiasis, the cholesterol content of the blood appears to play an important part in growth, both normal and neoplastic. Robertson and Burnett have shown that the rate of tumor-growth in rats can be materially accelerated by intravenous injections of cholesterol, but that, if the structure of the cholesterol molecule is changed (by transformation of "pure" cholesterol into acetyl-cholesterol or cholesterol chloride, that is into "changed" cholesterol) the tumors will no longer be affected. The writer[10-14] has repeatedly called attention to the importance of the blood cholesterol in connection with cell proliferation and was recently[14] able to show that radium treatment increased the amount of changed cholesterol in the blood while reducing the pure cholesterol. The mechanism of this effect of radium is still by no means clear (there seems to be some relation to the lymphocytic and leucocytic reaction), but it was observed that those patients who showed the greatest increase in difference between their pure and changed cholesterol, also appeared to derive the greatest benefit from the treatment. This observation seems specially significant in the light of Robertson and Burnett's investigations, but it is obvious that massed evidence will be required before we can hope to understand the mechanism involved in the relation between cholesterol metabolism and malignant growth. Further observations by many independent workers are therefore urgently needed. In this connection five points should be emphasized:

1. The nature of the test for cholesterol.
2. The importance of a uniform method for cholesterol determinations.
3. The source of the cholesterol intake.
4. The factors that influence the blood cholesterol.
5. The practical results to be expected in cancer research from the study of cholesterol metabolism.

Nature of the Test for Cholesterol.—Since cholesterol is a definite chemical entity and the test for cholesterol is based on purely chemical reactions, the condition under which determinations are made must control the values that are found in our determinations. In the literature dealing with the cholesterol content of human blood in health and disease, there exists at present a somewhat bewildering lack of uniformity as regards the values given by different writers. In many instances we find the most contradictory statements concerning the blood cholesterol values which accompany pathologic conditions. Such contradictory reports can undoubtedly be explained to a great extent by the varied technic used for the determinations:

1. The many methods used for the extraction of the blood cholesterol.
2. The fact that whole blood was used by some and only blood serum by others.
3. The relative strength or nature of the color standard in colorimetric determinations (it is well known that light shades can be matched more accurately than darker shades of any given color, and that the cholesterol standard and blood cholesterol generally have a slightly different tint).

4. The "setting" of the colorimeter for the standard, because of its influence on the relative depth of color of the standard solution.

5. The temperature at which the determinations were made; a relatively high temperature (35° C., suggested by Autenrieth) tends to produce lower values than does room temperature (20° to 22° C.), a fact to which I have called attention,[10] and which has since been corroborated by Bloor.[5]

6. The time during which the tests are allowed to "ripen."

7. The effect of daylight on the color reaction. Tests left in the dark assume a yellowish-green tone, whereas the same sample will be bluish-green when exposed to the light for the same length of time and at the same temperature. The color of the standard cholesterol always has a tendency to be more bluish (emerald green) than the blood sample and the respective shades can be more accurately matched if the tests correspond in tone.

Another important factor to which I also recently called attention is that there are two distinct types of blood, each with a different rate of color reaction; the rapid and the slow. The rapid type reaches its color maximum ahead of the commercial cholesterol standard and fades more rapidly than the latter. The slow type reacts less quickly but retains its color maximum longer. Unless this peculiarity is duly taken into account, especially when extreme representatives of either type are being tested, the values recorded will not be in accordance with the actual cholesterol content of the sample. Jaundiced patients usually have the very slow type of blood because of the bile derivatives present in the circulation. It has not been possible thus far to determine in what conditions the very rapid type of blood is most frequent; the rapid reaction was found in a good many cases of exophthalmic goiter, but the rapidity of the cholesterol reaction did not seem proportionate to the metabolic rate of the patient. In all our cholesterol determinations special attention has been paid to the rate of reaction of the test, which might introduce an element of error. Bloor has made the same observation and states that "the blood cholesterol behaves differently from the standard cholesterol." A permanent standard, would, therefore, undoubtedly offer many advantages, and will be discussed in connection with the need of a standardized, uniform method of procedure.

The blood constituents which are responsible for the different rate of color reaction are eliminated to a greater or lesser extent according to the method used for extraction. They may be looked on in a general sense as "changed" cholesterol, and are, in part at least, closely related to various bile derivatives.[12]

Importance of a Uniform Technic in Cholesterol Determinations.—It can not be emphasized too strongly that, unless comparable results are obtained by many observers, it will be impossible to establish the relation of cholesterol metabolism to pathologic conditions. The use of different methods for clinical observations is bound to result in conflicting findings and has undoubtedly been responsible for the conclusion that cholesterol determinations are of little practical value. In biochemic studies the use of various methods offers certain advantages, but for clinical observations a uniform method of procedure would seem a *sine qua non*. If we consider the clinical data supplied by hemoglobinometry, the truth of this statement will straightway be apparent. In hemoglobin

determinations the actual color value of the blood may be influenced by the presence of other blood constituents, but the fact remains that the hemoglobin test has proved a valuable asset to the clinician.

Bloor's methods, which have been used in all our determinations, seem particularly well adapted for clinical work, as they have two advantages, first, the extraction is both simple and complete, and second, the amount of changed cholesterol can easily be determined by means of parallel determinations with the Bloor I and Bloor II methods. Since systematic observations by these methods have already furnished data of clinical interest with regard to the effect of radium on the blood cholesterol,[13] it seems highly desirable that further independent observations should be made along these lines.

A detailed outline of the technic[20] used in our cholesterol determinations will be given in the hope that it may be of use to others working with cholesterol. The outline contains suggestions concerning a number of "pitfalls" to be found in cholesterol determinations, which we learned to recognize while determining the cholesterol content of more than 1500 individual blood samples. (See Tables I and II.)

The Source of Cholesterol Intake.—Cholesterol ($C_{27}H_{45}OH$) forms a constituent of every mixed diet. Our daily food is therefore a perpetual source of cholesterol intake. To compute a diet that is entirely free from cholesterol but containing the requisite number of calories, as well as the necessary amounts of protein, carbohydrates, fat, and mineral salts, might be a difficult task. Such a diet would probably be very unpalatable and it is doubtful, to say the least, whether it would prove beneficial in the end.[13] Milk, eggs, and butter for instance, which even when we do not use them in pure form, are yet to be found associated with most of our food when it is ready for consumption, would have to be eliminated completely. Table III shows clearly, however, that certain foodstuffs contain far more cholesterol than others.

Whether the body possesses the power of synthesizing cholesterol from cholesterol-free material is still a matter of dispute. Oatmeal contains no cholesterol, yet when a considerable amount of oatmeal is consumed the blood cholesterol values are markedly increased. This paradoxical phenomenon is still unexplained and so far as I know, we have no means at our command by which the problem can be solved, though tentative explanations have been suggested in Studies on Cholesterol IV.[13]

Since cholesterol metabolism appears to be a factor in malignant conditions, however, the cholesterol intake in our food would seem to deserve more attention than it has hitherto received. We know that in diabetes the metabolism of the carbohydrates is impaired and that the pancreas of the diabetic is less efficient than the normal pancreas. Joslin has shown that the treatment of diabetes constitutes one of the triumphs of modern medicine. By eliminating or restricting the use of the foodstuffs that the diabetic can not "handle," patients may be kept well and happy. Joslin gives the history of a man (Case 629, p. 559)[9] whose condition was complicated by tuberculosis and angina pectoris when he came under observation in 1913, but "who still leads an active life at the age of seventy-eight years, and has lately taken up politics." This patient had, however, been kept "under close supervision by a devoted nurse, thoroughly trained

in diabetes." If so much can be done for diabetics by safeguarding the weak spot in their metabolism, it seems rational at least to try to lighten the task of cholesterol metabolism for cancer patients by advising them to avoid foods that are rich in cholesterol.* The cholesterol content of the blood can be increased, even in a healthy person by the consumption of a great deal of meat (Fig. 1), and that eggs, with their high cholesterol content, are likely to have the same effect, is to be expected.

In diabetes chemical analyses supply the means by which we gauge the progress or the exacerbation of the patient's condition, but systematic observations and uniform methods had to be employed over a considerable period before prac-

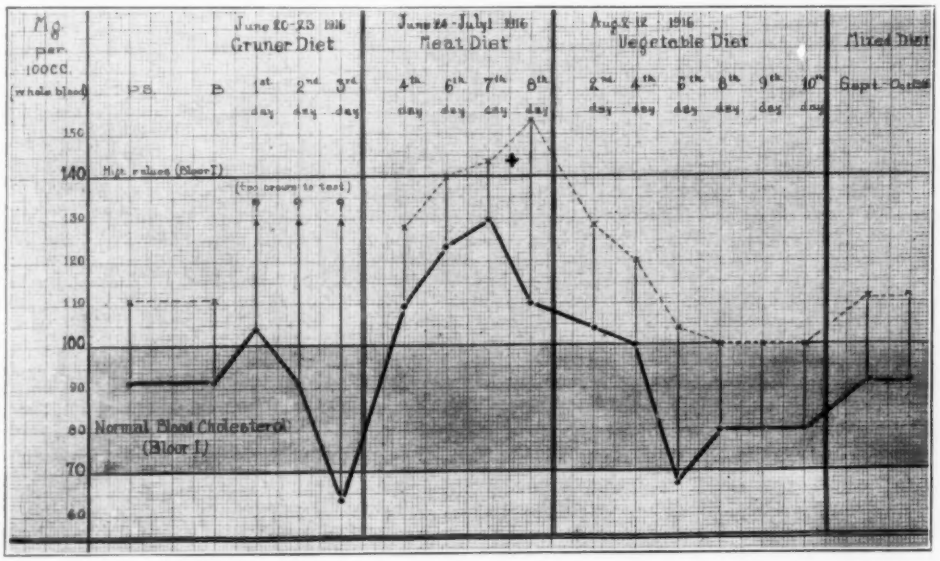

Fig. 1.—PS: The personal cholesterol standard of the writer from January to June, 1916. The tests were made at irregular intervals and showed practically no variations. B: The test taken the day before the experiment was started.

Grunner's diet: Milk, water, bread, and lettuce. The Bloor II values could not be determined; the tests were too brown-green because of a great amount of "changed" cholesterol, as shown by the rapid drop of the Bloor I values (Bloor I solid line, Bloor II dotted line).

Meat Diet: Meat was used exclusively for every meal, except for a very small piece of bread to "help the meat down" when the latter became very repugnant. + Attack of diarrhea, increasing Bloor II and lowering Bloor I.

Vegetable diet: Vegetables and fruit exclusively, tea and coffee as usual. Observe the drop of Bloor I values, and the satisfactory amount of "changed" cholesterol during this period as well as the gradual decrease of the changed cholesterol (vertical line) during the exclusive meat diet before diarrhea intervened, and that no diarrhea occurred on the vegetable diet, but that the blood settled down to a lower level.

Mixed diet: Meat twice a day, fruit, vegetables, tea and coffee, no alcohol; the usual type of ordinary mixed diet. Observe that the blood cholesterol returns to its former level.

tical results could be obtained; many factors besides the percentage of sugar in the urine had to be taken into consideration in order to reach the therapeutic successes that we observe today.

In carcinoma systematic observations on the cholesterol content of the blood by a uniform method, and in connection with dietetic and therapeutic measures

*Here again massed evidence will be required. At my suggestion a number of patients have reduced their cholesterol intake, apparently with beneficial results, but sufficient time has not elapsed to warrant definite conclusions. Blood cholesterol determinations and complete blood counts have been made to check up the condition of these patients and the results thus far may be looked on as promising. The details of the observations will be published later. Bulkley has advocated dietetic measures for many years and has recently again emphasized the metabolic origin of cancer.

would not only help to keep us informed, but also very materially further our understanding of the causes of malignant growth.

Factors That Influence the Blood Cholesterol.—The amount of changed cholesterol present in the blood is influenced by a number of factors. It is influenced to a marked extent by radium treatment, by the process of digestion (a factor that may, however, be easily eliminated by taking the blood before breakfast), by acute bacterial infections, and apparently by ulceration and hemorrhage. This shows that cholesterol metabolism is influenced directly by any process causing a reaction in the body, that is, by a process that tends to increase the metabolic rate. In myxedema an inversely proportional relation has

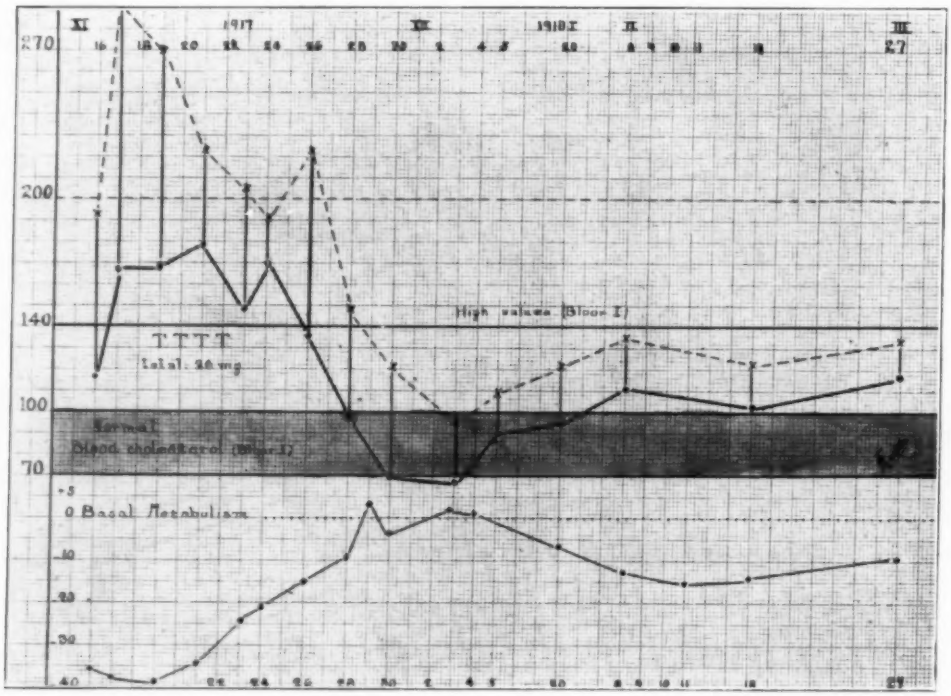

Fig. 2.—The relation between the rate of basal metabolism and the cholesterol content of the blood; observe that it is inversely proportional. The patient, a pronounced case of myxedema, went home perfectly well after the administration of the thyroid hormone (Kendall's thyroxin), 5 mg. at each dose.

been observed between the cholesterol content of the blood and the rate of basal metabolism[14] (Fig. 2). It will also be remembered that the process of spontaneous regression in malignant tumors, whether the tumors be grafted or of autogenous growth, is usually accompanied by ulceration, "sloughing" or hemorrhage; that radium treatment produces these symptoms as well as a "general reaction" in many cases; that acute bacterial infections (such as erysipelas, for instance) often exert a curative influence on the growth of tumors, and that the rate of basal metabolism is manifestly increased by all of these factors, as shown by the accompanying febrile reaction. Consequently recent bacterial infection, ulceration, sloughing and hemorrhages will have to be taken into account in the clinical interpretation of our cholesterol values. In the results obtained by Coley's treatment, the "reaction" plays a prominent part; its effect on the relation be-

tween pure and changed cholesterol in the blood would therefore seem well worth investigating.

In this connection I wish to call attention to the significance of Rhodenburg's[17] analysis of 302 cases of spontaneous recession of tumor in man. For the sake of convenience Rohdenburg's Group IA is given in tabular form. This group contains 43 cases (25 of carcinoma, 10 of chorioepithelioma and 8 of sarcoma) all showing complete recession, carefully controlled. The sequence in which the cases are given in Rohdenburg's article has been observed and the different types of tumors (Tables IV, V, VI) have merely been separated. The importance of the fact that these tumors disappeared, and that a patient was well and free from recurrence for a period as long as three and a half years in a case of melanosarcoma and of twenty-two years in a case of carcinoma, can hardly be overestimated. It would seem conclusive evidence that the human body is capable of waging a successful battle against malignant disease, for, in the instances quoted, this battle was fought and won under the most untoward conditions; in some cases the tumors were inoperable and the patients were sent home to die. Their condition would have justified the most pessimistic prognosis; none of them received radium treatment, and the operations that are recorded were for the greater part either "palliative" or "incomplete." Yet tumors, as well as recurrences, disappeared and gave no more trouble.

That the stimulation of the metabolic rate played some part in these recoveries, is suggested by the following: Among the cases of sarcoma, bacterial infection intervened in 4 of 8 cases; one case of chorioepithelioma, a "febrile convalescence of six days" is especially mentioned, in another the patient "had a temperature of 41°C. (106°F) for several days." No febrile reaction is reported in the carcinoma group that showed complete recession, but the reabsorption of the tumors themselves is likely to have stimulated the metabolic rate, to a certain extent, since the reabsorption of body proteins (exudates, hemorrhage) is almost invariably accompanied by a rise of temperature and the latter indicates a rise of the metabolic rate. That a too rapid reabsorption of protein material, by overdriving the organism as a whole, may become fatal to the patient is illustrated by Case 3 in Table III. In the other instances, however, reabsorption appears to have been less precipitous, and being more gradual, might have insured a continued stimulation of the metabolic processes by which the chemical conditions in the body were effectually altered. Be this as it may, the fact remains that in apparently hopeless cases the patient became "clinically well" (which after all is the main thing from the patient's point of view) and that they did so under conditions well calculated to increase the rate of their basal metabolism.

Unfortunately we do not possess at present any data obtained by modern methods on the metabolic rate in malignant conditions. I am beginning to collect such data but thus far only two observations have been available; in both instances the metabolism proved to be somewhat below normal. Two observations do not constitute much evidence and a metabolic rate that is only slightly below normal (8 and 11 per cent) may seem insignificant, but it will be conceded that even a slightly subnormal metabolic rate, existing over a prolonged period—half a lifetime maybe—may as effectually disturb the chemical con-

ditions under which the body cells have to exist, and influence the cells more potently, than a marked derangement of metabolism covering a short period. Systematic observations on the rate of basal metabolism in carcinoma before and after radium treatment in connection with the cholesterol content of the blood and the lymphocytic reaction (Murphy's "lymphoid defense") should therefore be looked on as valuable assets in the study of the cancer problem. It is obvious, however, that investigations along these lines can not be carried out by a single person; here again massed evidence must be obtained in order to furnish conclusive data.

The Practical Results to be Expected in Cancer Research from the Study of Cholesterol Metabolism.—There can be little doubt that the functional inefficiency of one organ may become the primary cause of perverted metabolism; no matter whether that organ has become inefficient after being "damaged" in some way, or whether it was originally inferior through faulty differentiation. The influence of thyroid inefficiency on the metabolic rate in myxedema, and the inadequacy of the pancreas and the chemical derangement it produces need only be recalled.

I have previously suggested[10] that the hereditary transmission of inadequate organs may be a primary factor in the etiology of malignant disease. Since we admit the hereditary transmission of external features (the family nose, the Hapsburg lip) and prove our belief in heredity by the attention devoted to our pedigreed stock, we can not logically deny that internal organs must also be subject to hereditary transmission. But if internal organs are transmitted thus, it follows that their inability to do their work properly must also become the cause of faulty metabolism and produce abnormal chemical conditions in the body. Nor is it to be expected that the composition of the blood should be normal under these circumstances, and, since the blood is the main source of food supply for all the body cells, the abnormal chemical composition of the blood is bound to affect the life of the cells, their structure, their development and their rate of proliferation.

In carcinoma we have not yet been able to recognize the organ that may be chiefly responsible for the type of faulty metabolism that can incite the cells to lawless proliferation. In diabetes, however, we know one organ at least that is unequal to its task, the pancreas. Numerous microscopic studies have failed to reveal any striking histologic changes in the pancreas of the diabetic; the gland seems to be merely functionally inefficient. Moreover, we admit that diabetes "has a tendency to run in families" (Joslin) and thereby indorse the fact of the hereditary transmission of the inadequate gland. By systematic observations on the chemical features that characterize the disease and which are, for the greater part at least, due to pancreatic inefficiency, we are now able to prolong the life or add to the comfort of diabetics to the extent described by Joslin. Had the same amount of energy that has been devoted to the study of chemical and metabolic details in diabetes, been similarly employed in cancer research, the verdict "that 90 per cent of those in whom carcinoma develops, succumb to the disease"[6] might never have been pronounced. Neither should it be forgotten that the chemical abnormalities observed in the blood of diabetics, high alkalinity, increased sugar content, and hypercholesteremia, are also found in car-

cinoma.[14] Moreover, cancer very frequently develops in diabetics (Robin, Joslin), whereas it is said that cancer patients rarely acquire diabetes. Possibly the latter statement might have been modified, if our chemical data had been collected with equal care in both diseases.

It would be obviously unwarranted, as well as foolish, to ascribe a common origin to conditions that differ so much in their manifestations as do diabetes and carcinoma; but a fundamental similarity may nevertheless exist between them; namely, the functional inefficiency of indivdiual organs; the dissimilarity of the organs that were primarily responsible would naturally determine the course of the diseases in either case.

The results obtained in the treatment of diabetes are sufficiently striking, however, to stimulate our efforts to discover the source of those metabolic disturbances that promote lawless cell proliferation. Blood cholesterol determinations, made systematically in conjunction with observations on the rate of basal metabolism and on other chemical constituents of the blood and urine, as well as the cytology of the blood, might reasonably be expected to further our knowledge in this direction. The study of the "lipoid" metabolism is as yet in its infancy, but its relation to carcinoma seems unquestionable in the light of Robertson and Burnett's investigations.

It is for this reason that determinations of the blood cholesterol, one of the chief blood lipoids, should have a place in cancer research.

TECHNIC

1. Extraction.—The ether-alcohol extract of the blood is prepared according to Bloor's direction, namely, 3 c.c. of blood taken from the cubital vein by means of a graduated syringe, are squirted directly into 75 c.c. of the ether-alcohol mixture, (3 parts 95 per cent alcohol to 1 part ether per 100 c.c.), well shaken to insure a fine division of the blood, just brought to the boil on the water-bath the bottle being shaken slightly all the time to prevent overheating, cooled under the tap for about five minutes, and then filtered. The bottles are filled up to 100 c.c. if the tests can be made immediately, otherwise they are merely corked, sealed with paraffin, and kept in a cool place to be filled up before the tests are to be made. This routine was adopted for the sake of greater accuracy, as the ether is bound to evaporate somewhat and thereby increase the concentration of the extract. Observations have shown that most extracts will remain unchanged for from one to six months; others, however, show variations in the relation of the changed and the pure cholesterol, due presumably to a disintegration of the cholesterol molecule, so that it is safer to make the tests as soon as possible after the blood has been drawn. We do not use citrate and have not had any trouble through clotting of the blood. Sodium citrate appears to affect the cholesterol values in some types of blood (no explanation can be offered for this observation, but the matter is being investigated); we have, therefore, discarded its use in order to insure similar conditions for all our tests. The extracts are kept in graduated 100 c.c. bottles, care being taken that no increase of concentration has occurred in samples that are to be retested after weeks or months.

For the Bloor I tests, 2 c.c. of sodium ethylate (3 gm. of metallic sodium dissolved in 100 c.c. of absolute alcohol) are added to each 10 c.c. of ether-alco-

hol extract; for the Bloor II tests, no sodium ethylate is added. We use 30 c.c. of the extract for each determination, so as to have enough material for three tests in each case. The extracts are measured into beakers and evaporated to dryness on a water-bath. As soon as they are dry the samples are taken off the water-bath, to avoid overheating, and left to cool for from fifteen to twenty minutes. The residue in the beakers (a faint yellow layer in the Bloor I, but hardly visible in the Bloor II samples) is then extracted three times with small quantities of dry chloroform. Any trace of water in the chloroform interferes with the subsequent color reaction. We have found it convenient to keep the chloroform in a wide glass bottle into which has been placed a quantity of calcium chloride; the chloroform is filtered before it is used. Each 10 c.c. of the ether-alcohol extract should provide 6 c.c. of chloroform extract when the three extractions have been added together, a total of 18 c.c. of chloroform extract being obtained when 30 c.c. of ether-alcohol extract is used per sample as in our determinations.

2. Color Reaction.—To 6 c.c.* of the chloroform extract are added 2 c.c. of acetic acid anhydrid and 0.2 c.c. of water-free concentrated sulphuric acid. We use ordinary test tubes, and shake the chloroform and reagents well to insure thorough mixing. We have found that the use of 0.2 c.c. instead of 0.1 c.c. as generally recommended, has the advantage of producing a tone of green that can be matched more readily, but does not influence the cholesterol values (the same amounts of the reagents are, of course, added to the cholesterol standard). Since the color reaction is due to oxidation, it seemed possible that the use of more sulphuric acid might increase the amount of changed cholesterol. This, however, is not the case. On the contrary, in a series of some 20 samples tested both with 0.1 and with 0.2 c.c. of sulphuric the amount of changed cholesterol was slightly higher with only 0.1 c.c. in some cases, though it was not altered in the majority of them. This observation is important, because in a series of determinations of the blood cholesterol in carcinoma of the cervix before radium treatment we had found, much to our surprise, that the number of patients whose blood gave equal values with the Bloor I and II tests was very small, whereas in the series previously reported,[13] including diverse localization of the disease, the percentage of equal values had been high (56 per cent). Since the use of more sulphuric acid lessens the changed cholesterol but certainly does not increase it, the behavior of the blood in carcinoma of the cervix (namely, the relatively big amounts of changed cholesterol) can not be due to our technic, but must be due to other factors. As most of the patients suffering from carcinoma of the cervix had a history of severe hemorrhage or of ulceration, these two factors would seem to influence the amount of changed cholesterol in the blood.

3. Technic Used in the Test.—After the reagents are added, the test tubes are left exposed to the light at room temperature (20° to 23° C.) for five minutes; this enables us to watch the rapidity of the color reaction as compared with the standard cholesterol and helps us to decide whether the blood sample is of the slow or of the rapid type. It also causes the tests to assume a tint of green that is more easily matched. After five minutes the tests are poured from the test tubes into the colorimeter containers. Colorimetric readings are made at a few

*Six c.c. tests were originally reccommended by Bloor. We are using them as this greater volume is more satisfactory in the large Duboscq colorimeter; the plungers do not get out of their depth in samples containing little cholesterol.

minutes' interval from 5 to 15 minutes after the reagents are added. The reading which persists at least four times is recorded as the maximum value. The cholesterol standard begins to fade in about twenty minutes[12] and for this reason we never use a standard that is more than fifteen minutes old. The reagents should be added to the standard and to the sample as nearly simultaneously as possible, and a fresh standard should be used for each sample. Table I has been found a great convenience in our determinations, as it gives the cholesterol values in milligrams per 100 c.c. directly from the colorimetric reading.

4. Permanent Standard Solution "NBM."—Lately we have made observations with a permanent standard, the color value of which corresponds with the cholesterol standard given in Table I. This standard solution and the pure Naphthol Green B Solution recommended by Gorham and Myers can only be called "permanent" in a restricted sense; the solution itself will keep unchanged in the bottle, even when exposed to light, but the portion of it used in the colorimeter and thereby exposed to fumes of the reagents, changes its tone and decreases in color value. This is especially true if the closed Kober colorimeter is employed. Those who work with the latter instrument will have observed that a strong odor of acetic anhydride is retained in the instrument for some time after the tests have been taken out. As our containers and plungers were cleaned with special care and washed, first in a strong solution of sodium bicarbonate, then repeatedly with distilled water to remove all trace of acid, actual contact with acid can not be responsible for this deterioration of the Naphthol Green B, or of the NBM standard solution, which will, of course, result in inaccurate readings if the standard is looked on as truly permanent. We have found, however, that satisfactory and reliable results are obtained if the standard solution is renewed after each two or three samples have been tested. As in all our observations parallel determinations are made with two colorimeters, a standard cholesterol solution being used in one of them, while the reliability of the aqueous standards was being checked up, it was, of course, relatively easy to discover when the aqueous solution began to "go off." For those who only use one colorimeter, we would recommend changing the aqueous standard fairly often to insure the greatest accuracy. Even so the use of the aqueous standard will be found to be both convenient and economical. The NBM standard solution has a decided advantage in our estimation when compared with the pure Naphthol Green B, inasmuch as the latter is yellow green in tone and is much more difficult to match to the more bluish green of many of the blood samples, especially if the tests are left exposed to the light during the process of ripening as I have recommended.

Formula of the NBM Standard Solution.—NB stock solution; Naphthol Green B, 0.5 gm. to 100 c.c. of distilled water (0.5 per cent solution); M stock solution; methyl blue, 60 mg. to 100 c.c. of distilled water (0.06 per cent solution).* NBM standard solution: 1 c.c. of NB stock solution and 1 c.c. of M stock solution to 200 c.c. of distilled water. Stock solution NB should be added first to the 200 c.c. of distilled water, well stirred or shaken, after which stock solution M is added; if both stock solutions are mixed before dilution a

*Our Stock Solutions were made up in December, 1917, and have been found to be unchanged by testing them at intervals of several months against pure cholesterol standards up to the present, January, 1919. They have been exposed to the light all the time.

precipitate is apt to form which can be prevented if the naphthol green is diluted before the methyl blue is added.

In Table II a series of tests is shown which were made to ascertain the reliability of the NBM standard. If we consider that the figures given in Table II represent independent readings by three observers, and that the reaction for cholesterol is a progressive reaction in which the color formation may be slightly more rapid in some blood samples than in others (although no "very slow" specimens are included in this series) the element of error in the readings is very small, on an average of 3 mg. per 100 c.c.

The fact should be taken into account that some 1500 individual blood samples have thus far been tested by me and my two helpers, which represents a total of between eighty and ninety thousand readings, six tests being used for each sample and about ten readings for each test; consequently the opportunity to train our color vision has not been lacking.

SUMMARY

1. The test for cholesterol is not a diagnostic test, but furnishes information concerning cholesterol metabolism; it will therefore furnish information about the disturbances of cholesterol metabolism connected with cholelithiasis and carcinoma, for instance.

2. Cholesterol metabolism is influenced by the rate of basal metabolism; it was found to be inversely proportional to the latter in myxedema.

3. The reaction for cholesterol is a purely chemical reaction; technical procedures play a prominent part in the results obtained. Consequently the method of extraction and the conditions under which the color reaction takes place determine the values found in blood cholesterol determinations.

4. In clinical work, intended to promote our knowledge concerning the relation between the cholesterol content of the blood and pathologic conditions, a uniform method of procedure should be adopted for cholesterol determinations, since this alone will insure comparable findings. A detailed account of the technic used in our determinations is given, because this technic is based on the determination of more than 1500 individual blood samples, which enabled us to recognize and eliminate many elements of error in the course of our work.

5. The cholesterol content of the blood is influenced by a number of factors, the nature of the diet, the rate of basal metabolism, radium treatment, bacterial infection (ulceration, infectious disease), and hemorrhage. These factors should be duly considered in the clinical interpretation of blood cholesterol values. The cholesterol content of the blood is intimately related to lipoid metabolism and the blood cholesterol can be influenced by dietetic measures.

6. It is known that the inefficiency of an individual organ can give rise to a particular type of metabolic disturbance; inefficiency of the thyroid results in myxedema, pancreatic inefficiency in diabetes.

7. There can be little doubt that an intimate relation exists between disturbances of the cholesterol metabolism and the lawless proliferation of cells that we observe in carcinoma, although the organ that is initially responsible for these disturbances has not yet been recognized.

8. The results obtained in the treatment of diabetes show what can be achieved in the face of organic inefficiency, and these results have been obtained

by means of chemical investigations and by dietetic measures calculated to counterbalance the inefficiency of the pancreas, whereas the histologic study of the latter did not materially advance our knowledge in the treatment of the disease.

9. The fact that in a number of carefully controlled cases, the tumors receded and the patients became "clinically well," although their condition had been pronounced "hopeless," constitutes proof positive that the human body can wage a successful battle against malignant disease.

TABLE I

CHART FOR BLOOD CHOLESTEROL DETERMINATIONS

GIVING THE CHOLESTEROL VALUE IN MG. PER 100 C.C. DIRECTLY FROM THE COLORIMETRIC READING

Standard: 0.4 mg. cholesterol to 6 c.c. of chloroform.* (This concentration harmonizes best in color with the value found in human blood). The NBM permanent standard is equally satisfactory (for formula see text).

Set at: 10. mm.—**Colorimeter:** Duboscq and Kober.

Cholesterol values in mg. per 100 c.c. of whole blood

mm.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.
	1332	666	440	333	267	222	191	167	148	133	121	111	102	95	89	83	78	74	**70**	67
mm.	mg.	mg.								mg									L. N.	
0.1	1210	636	430	326	261	218	188	164	146	132	120	110	102	95	89	83				
0.2	1110	603	417	318	259	216	186	163	145	131	118	109	101	94	88	82				
0.3	1020	580	410	310	251	212	183	162	144	129	117	108	**100**	93	88	82	77	73	69	66
													H. N.							
0.4	950	555	392	304	249	208	180	159	142	128	117	107	99	93	87	81				
0.5	895	533	380	296	241	204	177	157	**140**	127	116	107	99	93	87	81	76	72	68	65
									high											
0.6	840	515	370	290	238	202	175	156	138	126	115	106	98	92	86	80				
0.7	785	495	360	284	234	**200**	173	154	136	125	114	105	98	91	85	80	75			
						except														
0.8	745	477	352	278	230	198	171	152	136	124	113	104	97	91	85	79				
0.9	705	460	341	272	226	194	169	150	135	123	112	103	96	90	84	79	74	72	67	76
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.

mm.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.
	63	60	58	55	53	51	49	48	46	44
0.5	61	59	57	54	52	50	48	47	45	43
	mg.									mg.

Values below 63 mg.: rare in human blood but usually found in goat's blood.

mm.	40.	45.	50.	60.	70.	80.
	33	30	27	21	19	17
	mg.					mg.

These values do not occur in blood but were used in color determinations of "bile derivatives."

The designation of "normal" (70-100 mg. per 100 c.c. of **whole** blood) is based on the use of The Bloor I. Method, with the "standard" and setting of the colorimeter indicated above. Values between 100-140 mg. usually were found to occur in inflammatory conditions (digestion of course being excluded for every test): we refer to them as "increased."

Values between 140-200 are referred to as "high" values with the Bloor I. Method. Values over 200 mg. were only observed in a few cases so far (3 cases of myxedema, one Ca) they have therefore been called "exceptional" in our determinations.

In carcinoma "high" values are the usual finding, though "increased" and (rarely) normal values have been observed. "Equal values," viz., identical with the Bloor I. and II. method were found in 56 per cent of the cases of carcinoma who were to have radium treatment.

After Radium treatment the **Bloor I. values** were found to **drop,** whereas the **Bloor II. values increased,** causing the "equal values" to disappear. **No** "equal values" have been found so far in **non**-malignant cases.

N. B.—It should be remembered that the **cholesterol test** is **not a diagnostic test;** it is merely a "clinical" test, like the test for albumen in the urine.

*Stock Solution (A) = 200 mg. of cholesterol in 100 c.c. of pure chloroform.
Standard " (B) = 10 c.c. of A made up to 150 c.c. with pure chloroform.

10. Investigations tending to elucidate the factors that connect lipoid metabolism with malignant proliferation, may enable us to do as much for cancer patients in the future as we can already do for diabetics. Massed evidence collected by means of uniform procedures will be needed to attain this end. Blood cholesterol determinations are a means by which our knowledge concerning lipoid metabolism can be increased; hence they deserve a place in cancer research.

TABLE II

TESTS MADE WITH NBM PERMANENT STANDARD

1. "Standardizing" of the Duboscq and Kober colorimeter against each other with ordinary cholesterol standard.
   One side of the colorimeter is used exclusively for the standard and marked S.
   Double samples of the standard cholesterol are prepared and one of these is used as "unknown," in both the Duboscq and Kober.
   Standard set at 10 mm., "unknown" reads 10 mm., 3 times by three observers.
   This shows that both Kober and Duboscq give identical readings.
2. Duboscq: Cholesterol standard 5 to 10 minutes old, set at 10 mm.
   NBM used as "unknown."
   Readings: 10.1 mm. 10. mm. 10.; 9.9 mm. 10. mm. 10.1; 10.1 mm. 10 mm. 10. mm. = 133 mg. 132 mg. 135 mg. 133 mg. 132 mg. 133 mg.
3. Kober: Procedure as above, readings vary within same limits, between three observers.
4. Kober: NBM standard set at 10 mm.
   blood sample as "unknown."

Sample A		Duboscq: Cholesterol standard at 10 mm. blood sample used as "unknown."
Bloor I	20 mm. 20 mm. 19.9 mm. 19.9 mm. = 67 mg. 67 mg.	.....19.9 mm. 19.9 mm. 19.9 mm. 20 mm. = 67 mg. 67 mg.
Bloor II	11.3 mm. 11. mm. 11.1 mm. 11. mm. = 117 mg. 121 mg. 120 mg. 121 mg.	.....11. mm. 11.2 mm. 11. mm. 11. mm. = 121 mg. 118 mg. 121 mg. 121 mg.
Sample B		
Bloor II	9.2 mm. 8.2 mm. 8.8 mm. 8.8 mm. = 145 mg. 152 mg. 152 mg. 152 mg.	.....8.1 mm. 8.8 mm. 8.7 mm. 8.8 mm. = 164 mg. 152 mg. 154 mg. 152 mg.
Sample C		
Bloor I	11.7 mm. 11.7 mm. 11.7 mm. 11.7 mm. = 114 mg. 114 mg. 114 mg. 114 mg.	.....12 mm. 11.9 mm. 11.9 mm. 12 mm. = 111 mg. 112 mg. 112 mg. 111 mg.
Bloor II	9. mm. 8.9 mm. 8.8 mm. 8.9 mm. = 148 mg. 154 mg. 152 mg. 150 mg.	.....9.1 mm. 8.8 mm. 9.1 mm. 8.7 mm. = 146 mg. 152 mg. 146 mg. 154 mg.

Sample D, A mixture of all the remnants of Bloor I and Bloor II tests made that morning of which the cholesterol value was naturally beyond guessing.
10 mm. 10.1 mm. 10.2 mm. 10.1 mm. = 133 mg. 132 mg. 131 mg. 132 mg.
.....10 mm. 10 mm. 10.1 mm. 10 mm. = 133 mg. 133 mg. 132 mg. 133 mg.

TABLE III

CHOLESTEROL VALUE OF FOOD

(Mg. Cholesterol per 100 c.c. or 100 gm.)

THREE GM. SAMPLE	METHOD	
	BLOOR I	BLOOR II
Yolk of egg (raw)	888	888
Yolk of egg (hard boiled)	888	888
Butter	157	298*
Chicken (roasted breast)	127	127
Beef (raw)	53	53
Beef (roast)	63	63
Beef (smoked, salted, dried)	60	83**
Fish, black bass	83	83
***Mushrooms (plain cooked, agaricus campestris)	140	102
Mushrooms (stewed in butter, another batch of agaricus campestris)	70	60
Cream (raw)	61	61
Milk (raw)	28	28
Oatmeal ("porridge" boiled)	0	0

*Probably due to some coloring matter, eliminated in Bloor I by sodium ethylate.
**Reddish tone of ether alcohol extract suggests coloring matter, eliminated in Bloor I by sodium ethylate.
***Mushrooms contain phytosterol, a substance allied to cholesterol, but give the green reaction with the usual reagents, the lower value with Bloor II is an unusual phenomenon, for which no explanation can be offered; probably due to the phytosterol itself.

TABLE IV

SARCOMA : 8 CASES

*GROUP IA COMPLETE RECESSION. CAREFULLY CONTROLLED

DESCRIPTION OF FIRST FINDINGS		TIME ELAPSED UNTIL SECOND FINDINGS	
Melanosarcoma back-axilla	Primary tumor involvement of axilla removed by operation	3½ years	Still free from recurrence
Round cell sarcoma, chin cervical glands	Chin, operation, recurrence in right and left cervical glands. Left cervical removed	No time given	Spontaneous recession after erysipelas of remaining nodules
Round cell sarcoma, axilla	Operation, recurrence, incomplete curetting	13 months	No recurrence, remnants of tumor disappeared after erysipelas
"Sarcoma," axilla	Many metastases; pregnancy followed by abortion	No time given	Tumors disappeared, died six weeks later
Fibrosarcoma skull	Secondary deposits, partial removal	No time given	All tumors disappeared
Round cell sarcoma of the breast	Many metastases in subcutaneous tissues	No time given	All neoplastic deposits disappeared after acute infection of lung (pneumonia?)
Lymphosarcoma behind the ear	Inoperable	No time given	Complete recession after inoculation with erysipelas

One case of melanosarcoma showed regressive changes but "changes did not progress to the degree of clinical manifestation."

*Rohdenburg, G. L.: Fluctuations in the Growth Energy of Malignant Tumors in Man, with Especial Reference to Spontaneous Recession, Jour. Cancer Research, 1918, iii, 193-225.

TABLE V

CHORIOEPITHELIOMAS.—10 CASES

*GROUP IA COMPLETE RECESSION. CAREFULLY CONTROLLED

DESCRIPTION OF FIRST FINDINGS	TIME ELAPSED UNTIL SECOND FINDINGS	
Fundus uteri perforated, bladder involvement, part of tumor attached to bladder	16 months	Patient well, no mass palpable (febrile convalescence of 6 days)
Uterine wall perforated, pelvic involvement; incomplete removal	1 year +	At autopsy no recurrence was demonstrable
Invasion of uterine muscle; curettage	1 year	Patient well
Cherry-sized tumor, posterior wall, tumor removed	10 weeks 1 year	Curetting, no abnormal histology Patient clinically well
Nodule in vagina removed, recurrence 2 weeks later, suppuration and disappearance	10 months	Patient well
Nodules in vaginal wall and uterus, 2¼ years after hydatidiform mole had been delivered; nodules removed	10 months	No evidence of recurrence
Chorioepithelioma, curetted, temperature 106 (41° C.) for several days.	29 days later	Second curetting, negative Subsequently bore two healthy children
Abortion at 7½ months, curetted Metastases shortly after. Sent home to die (case Hörman)	1 year	Tumor and metastases disappeared Subsequently normal pregnancy

In the two remaining cases no malignancy was found after curettement in serial sections of the uterus removed by hysterectomy only a short time afterwards; consequently they hardly constitute evidence of regression.

*Rohdenburg, G. L.: Fluctuations in the Growth Energy of Malignant Tumors in Man, with Especial Reference to Spontaneous Recession, Jour. Cancer Research, 1918, iii, 193-225.

TABLE VI

CARCINOMA—25 CASES

*GROUP IA COMPLETE RECESSION, CAREFULLY CONTROLLED

LOCATION OF FIRST FINDINGS	TIME ELAPSED UNTIL SUBSEQUENT FINDINGS	
Stomach; Advanced, widespread peritoneal metastases, ascites	2½ years	Autopsy, no local recurrence, metastases, and ascites absorbed
Tonsil-tongue: So extensive as to be inoperable. Not treated, mouth wash, sent home to die	18 months	No trace of original tumor
Breast: Amputation, 1904, recurrence, 1906, August (x-ray), practically moribund until December, 1906	Dec. 28, 1906	Sudden, "overnight" absorption of entire area, died three weeks later
Breast: Amputation, recurrence in scar, metatases in liver	Next few years	No treatment, recurrences disappear, subsequently "entirely recovered"
Uterus: 1 fundus, 2 cervical, all inoperative; palliative cautery, curetting	6 to 7 years	All clinically well
Breast: Amputation, 2 years after recurrence in scar, metastases uterus, inoperable	3 years	Uterus normal, nodules in scar have disappeared
Uterus: 2 cases, inoperative, palliative curetting and cautery	4 to 6 years	Clinically free from all evidences of carcinoma
Intercostal: Recurrence of breast cancer, inoperable	7 years	Completely receded, free from recurrence
Uterus: Inoperable, palliative curettement, cauterized	4 years	Well, uterus normal condition
Maxilla: Recurrence 3 months later. No treatment	3 months	Recurrence completely absorbed
Broad ligaments: Infiltrated, incomplete removal of uterus	7 years	No evidence of malignant growth
Stomach: Inoperable, metastases to mesentery (exploratory laparotomy)	9 years	Perfectly well
Uterus: Cervix, curetted, cautery	11 years	Well, no evidence of tumor
Uterus: Curettement, cautery	11 years	Well, no evidence of tumor
Uterus: Radical operation refused, epithelioma cervix	22 years	Well, no evidence of tumor
10 remaining cases no definite data as to time given, patients well		

*Rohdenburg, G. L.: Fluctuations in the Growth Energy of Malignant Tumors in Man, with Especial Reference to Spontaneous Recession, Jour. Cancer Research, 1918, iii, 193-225.

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LABORATORY METHODS

NOTES ON THE ISAACSON METHOD FOR ESTIMATING GLUCOSE*

By Elisabetta C. Pennell, M.S., Santa Barbara, Calif.

THE rapid colorimetric method for determining glucose in urine, which was published by Victor I. Isaacson† was tried out in this laboratory with unsatisfactory results. Determinations were made on two standard glucose solutions and on twelve diabetic urines, both by this colorimetric method and by Fehling's volumetric method. The results obtained are tabulated below:

SOLUTION USED	AMOUNT USED	STANDARD FOR COMPARISON	RESULT ISAACSON'S	RESULT FEHLING'S	NO.
2.5% Glucose Solution	½ c.c.	B	5.2 %	2.5 %	1
Urine M 1/20]19	1 c.c.	A	0.5 %	2.38%	2
" P 1/14/19	1 c.c.	A	0.96%	0.88%	3
" P 1/16/19	1 c.c.	A	0.58%	0.88%	4
" P 1/17/19	2 c.c.	B	1.09%	0.78%	5
" R 1/15/19	1 c.c.	B	2.46%	3.12%	6
" R 1/19/19	1 c.c.	A	0.1 %	2.98%	7
" P 1/15/19	1 c.c.	C	1.25%	0.68%	8
" P 1/19/19	1 c.c.	A	0.00%	0.58%	9
" R 1/16/19	1 c.c.	B	1.25%	1.67%	10
" D 1/20/19	3 c.c.	A	0.00	0.4 %	11
" P 1/18/19	1 c.c.	A	0.00	0.71%	12
" P 4/18/19	3 c.c.	A	0.15	0.89	13
1% glucose solution	1 c.c.	A	0.9%	0.96%	14

These figures show a startling discrepancy. The chief difficulty for any one unacquainted with this method is in deciding the amount of sample to be used, and the standard required for comparison. The author of the method states that for percentage less than one, 3 c.c of the specimen must be used. In a determination such as (11), 3 c.c. were used, but the color of the filtrate was so dark that when diluted to compare with A, the darkest of the standards, the percentage was so low that when divided by 3 the result was zero or less. With high concentration of sugar it frequently happens that in sufficiently washing the filter paper so much water was required that the resulting solution was too light to compare with any of the standards. It is difficult to judge by a qualitative test whether the percentage of sugar is 0.8 or 1.2, for instance, so that the wrong amount of urine is frequently used, and either the resulting percentage is incorrect, or a repetition of the determination is necessary. This is more time consuming than the usual volumetric methods.

*From the Laboratory of the Memorial Laboratory and Clinic, Santa Barbara, Calif.
†See Journal of Laboratory and Clinical Medicine, Feb., 1918, iii, 299.

A colorimetric method in which neither the standard nor the unknown can be moved up and down, so as to compare first with lighter and then with darker color, is necessarily inaccurate. It is found that as much as 3 c.c. of water may be added to dilute the color of the unknown in this method without making any change perceptible to the eye. Another serious source of error is the necessity for covering the tube with the thumb, or a cork, to mix the contents, so that some of the color is almost always lost.

The Sahli hemoglobinometer, for instance, is an apparatus of this type, but with it no attempt is made to read closer than 5 degrees. If a colorimeter was made with one standard and one tube for the unknown which was graduated from 0.5 per cent to 9 per cent by 0.5's it might prove more successful than the one under discussion. A colorimeter of the Hellige type would seem even more feasible. The unknown in such a determination, if too dark could be diluted half, as in phenolsulphonephthalein determinations.

The determinations above reported were made with uniform care by one laboratory worker, and the solutions prepared quantitatively. The standard solutions were made of Kaulbaum's glucose.

A MODIFIED PORTABLE ARTIFICIAL RESPIRATION OUTFIT*

By John A. Higgins, Chicago, Ill.

IN REVIEWING the literature on the subject pertaining to artificial respiration machines, valves and methods of applying, a large number of articles on the subject can readily be referred. Fells,[1] Jackson[2] Hanzlik,[3] Gates,[4] Meltzer,[5] Meyer,[6] Hoyt,[7] Hall,[8] Sollmann,[9] Stewart and Rogoff,[10] Gessel and Erlanger,[11] Brodie,[12] Brunton,[13] Schafer,[14] Ludwig,[15] etc., have each endeavored to advance the subject by designing some type of an apparatus or method of procedure.

When the medical students begin to receive their training in the physiologic laboratory, pertaining to operations on the mammalian subject, they are seldom called upon to perform operations of a major character. The laboratory outline may possibly call for a dissection and ligation of the carotid artery or arteries, vagus nerve, femoral vein, sciatic nerve, the recording of blood pressure, etc., in which cases interference with voluntary respiratory movements are, as a rule, avoided. Since we can not predict, however, the ability of the student anesthetist or the susceptibility of the mammalian animal to the anesthetic administered, voluntary respiratory movements may at any period of the experiment cease to continue. In such instances, and where the heart is performing its function normally, the logical procedure would be to stop the anesthetic and apply artificial respiration.

The question involved at this period of training is whether the medical students should be allowed to familiarize themselves with an elaborate or complicated artificial respiration outfit, which they may be unable to have access to

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in their future career or whether it should be advisable to acquaint the students with a portable artificial respiration outfit that could be, with little inconvenience, had in the experimental laboratories, hospital operating room, or on the outside in general practice.

I am inclined to favor this last view, especially so with the beginning medical students. After a preliminary training on the simpler types of artificial respiration outfits or methods of procedure more elaborate or complicated outfits or methods can, of course, then be studied.

Elaborate types of artificial respiration outfits, while they may give perfect satisfaction and be very valuable within themselves, are as a rule aimed for use in the experimental laboratories, or possibly for use in an operating room. In such places electric energy or compressed air, from a power plant, is generally accessible for applying in connection with the artificial respiration outfit adopted. Unfortunately, however, suitable energy, except hand or foot power, can not be had in numerous instances. This latter view would perhaps hold general in emergency cases where resuscitation on the human is essential to retain life. Such views on this subject are perhaps already considered by a number of the laboratory instructors. Accordingly they often utilize the hand or foot bellows as best they can in cases of emergency. To make use of the hand or foot bellows independently does not, however, produce the intermittent interruptions desired. Some device or valve may then be used in connection to produce the desired effect.

Fells,[16] and Stewart and Rogoff,[10] have brought to the attention of the medical profession a valve, as previously referred to, for producing the desired interruptions. Whether Fells had in view the principles later involved in the Stewart and Rogoff type of valve, I am unable to state definitely. Fells had also, in connection with his type of valve, two side inlets. One of the inlets was to accommodate administering ether vapors in case a tracheotomy was performed, and a second smaller inlet to accommodate a rubber tube leading directly from an oxygen gas tank. Now while the Fells type of valve may be of value from an experimental point of view, I would be inclined to question whether the extra side inlets are advisable, especially where resuscitation on the human is to be applied.

Where Fells aims on performing a tracheotomy he thus limits the use of his valve to the medical profession. While Fells is perfectly justifiable in keeping such a point in view, yet it is quite possible to eliminate the larger side outlet to his valve and still be able to administer ether vapors via Woulff bottle beyond the valve. Contrary to his view, other writers advocate that the layman or first aid man should not hesitate in applying some form of artificial respiration, even in the absence of a physician, where life is involved at a critical period. This critical period rarely exceeds more than ten minutes after voluntary respiratory movements cease.

Ordinary air contains about 21 per cent oxygen, of which about one-fifth, under ordinary conditions, of the oxygen inhaled is absorbed. This would therefore seem a reason to eliminate the inflation of the lungs with pure or nearly pure oxygen, if no more oxygen is absorbed than by breathing pure air. To dispense with both side inlets from the Fells type of valve will bring us to a point

of principle resembling the Stewart and Rogoff type of valve. The Fells type of valve would still seem to offer as an advantage a sort of safety to check too much pressure accumulating in the distensible bag which is placed in the air circuit. Stewart and Rogoff offer as an advantage a sort of a thumb screw safety to prevent the possibility of excessive pressure inside the face mask, together with the valve being attached to the face mask to cut down on excessive dead space.

Feeling still that there was an opportunity to make certain modifications for the good of the subject, I have acted accordingly. These modifications were made possible by taking a Fells type of valve and cutting out the two side inlets. Add on the rubber bumper as shown at *K* Fig. 1 to eliminate friction or wear

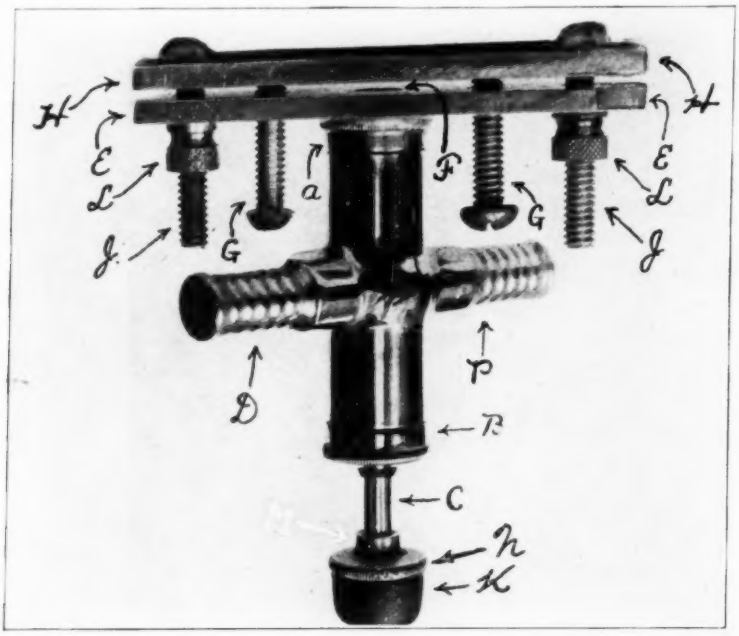

Fig. 1.—Showing view of attachment for combining with a Fletcher portable foot blower to produce an intermittent artificial respiration.

on metal piece *N*. The barrel head at *A* was then soldered to a solid piece of brass which measured 3¾ by 1⅛ by 3/16 inches as shown at *E*. Drill through this piece of brass, to correspond with the center of the barrel head *A*, a ⅜-inch hole *F*. About 13/16 of an inch, and in opposite directions from the center of the ⅜-inch hole, drill two holes through the brass piece and thread them to accommodate 16/18 screws *G*. These screws at *G* measure 1¼ inches over all and are used to regulate the stroke of downward movement on the foot bellows.

A second piece of metal *H*, measuring the same as piece *E*, is set flat against piece *E*. Drill a hole at each end, drilling through both pieces with a 9/32-inch drill, about ⅜ of an inch from the ends. These holes are to accommodate the slipping through of the 1¾-inches long by 16/18-inch threaded section of the screws *J*. On the threaded section of screws *J* are lock nuts *L*. These lock nuts

are used to fasten the valve to the treadle of the foot bellows as shown in Fig. 3. The bellows here used is a Fletcher portable, number 10-B, with an 11-inch in diameter reservoir. The tubes *D* and *P* (Fig. 1) have a $\frac{3}{8}$-inch opening and are connected to the air system as shown in Fig. 3. One of these tubes connects the outlet of the bellows reservoir to the valve. The second tube connects the valve to either a face piece or pharyngeal tube for human or a tracheal cannula, via ether vapors, in experimental mammalian work. With this outfit there is no need of any other special valve beyond the attachment itself.

The plunger used in connection is partly visible at *C*, *N* and *K*, Fig. 1, or fully visible in Fig. 2, except for the lower barrel head at *B*. The slot *E*, Fig. 2, is used in connection with an elevated section inside the valve barrel and to pre-

Fig. 2.—Showing view of the plunger.

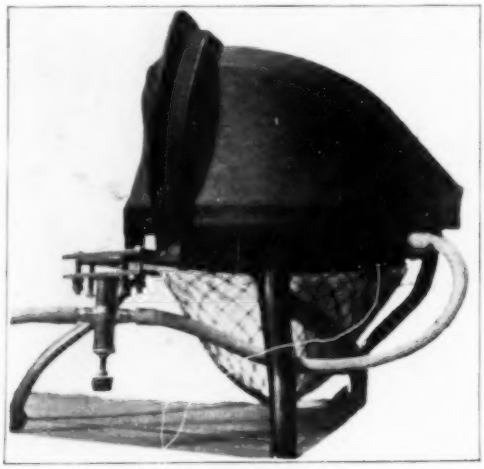

Fig. 3.—Showing view of a Fletcher portable foot blower with attachment attached and connected for producing intermittent artificial respiration.

vent rotation movements. The opening at *G* passes fully through the plunger, while the opening at *F* terminates at the end of the plunger at point *H*. A coil spring in the upper section of the valve barrel is used to keep the plunger under an outward tension.

The outfit as ready for practical use, except for the face mask or pharyngeal tube, is shown in Fig. 3. Upon forcing the bellows treadle downward, the valve being closed against air from the bellows and to the point where the lower section of the plunger hits the ground surface, the air is being transferred from the upper bellows chamber to the lower reservoir. As we continue the downward stroke, after the plunger head first hits the ground surface, the plunger is forced up into the valve barrel piece to a point where the barrel head *B* (Fig. 1) hits the shoulder *M*. When this point is reached, it brings opening *G* (Fig. 2) opposite the tube openings at *D* and *P* (Fig. 1) and thus permits the air, already placed under tension in the bellows reservoir, to proceed toward inflating the

lungs. Then upon releasing the tension from the bellows treadle the coil spring forces out the plunger to its regular position, closes the system of air from the bellows reservoir to the valve, and opens the system of air from the lungs to the outside via openings *D, F* (Fig. 1) and *F, H* (Fig. 2). This thus permits the lungs to collapse. At the same time the upper chamber of the bellows is filling up for a succeeding expiration.

It will now be noted that in this outfit I eliminate the distensible or interposed extra rubber bag between the bellows and the valve as found in other outfits. It is made possible to limit the intermittent expirations by regulating screws *G* (Fig. 1) and thus relieve considerable danger of excessive lung pressure. The expirations from the bellows reservoir can be maintained to flow with practically very little, if any, variations. The outfit is portable and can be kept connected. The face piece or pharyngeal tube can be connected at a short distance from the valve and thus eliminate dead space. The outfit can be worked either by foot or by one hand leaving the other hand free to look after the face mask or pharyngeal tube. The outfit should, however, rest on either a board or solid ground to insure uniform expirations as a sandy or soft ground may cause variation.

Since we do not ordinarily utilize our lungs to full capacity, I would here suggest setting a standard for the outfit. For example, record the average amount of air expired at each expiration from a number of adults. Sum up this total, and find an average lung capacity. Regulate the artificial respiration outfit accordingly for general use in emergency cases. There may be marked differences between male and female adults, but in such cases it might be advisable to have two or more such outfits so regulated. The outfit should be worked to correspond with normal breathing. Before applying artificial respiration see that the mouth is free of any foreign body, keep the tongue pulled forward, and see that the plunger in the valve reacts normally. While this outfit should not interfere with training along the Shäfer prone method, still the outfit is perhaps as safe as can be trusted in the hands of the layman.

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THE USE OF FLOCCULE-FORMATION REACTIONS TO CORROBORATE THE WASSERMANN TEST

By Ross J. MacCann, New York City

AMONG the diagnostic methods that are used for the detection of syphilis, the original Wassermann technic with some of its varied modifications, is the one that is mostly used in commercial laboratories. Many of these laboratories use two antigens, cholesterinized and noncholesterinized antigens, or an alcoholic extract of syphilitic liver; while a few use a third antigen, Noguchi's acetone-insoluble lipoid extract. Some laboratories use the Hecht-Weinberg, one of Kolmer's modifications, or the Hecht-Gradwohl method in conjunction with the regular Wassermann, so that anticomplementary findings may be reduced to a minimum.

The use of more than two antigens lengthens and complicates the tests; in the Hecht-Weinberg modification the serum should be used as soon as possible after the specimen is taken, utilizing as it does the complement and amboceptor in the patient's serum, which is perfectly feasible where Wassermanns are being done daily, but not where they are being done once or twice a week; while the Hecht-Gradwohl modification is inclined to be rather cumbersome, especially where many tests are to be made in a day; and in Kolmer's methods, two of them are almost similar to the Wassermann, while the other two consume a great deal of time and much material. All these modifications are excellent as corroborative reactions with the regular Wassermann, but where the time is limited, a shorter and yet reliable method would be more advantageous, and helpful in doubtful cases.

These doubtful or anticomplementary findings would not be commented on to any great degree, in hospitals, clinics, etc.; in a commercial laboratory they are a source of friction between laboratory and physician, and looked upon as a breach of contract, by the patient involved. He has paid his fee and naturally looks forward to a positive or negative report; when they receive a report with the explanation that their blood differs somewhat from others, and that the result is a doubtful one, it is a difficult task dispelling the patient's idea that it was due to carelessness, and not to the anticomplementary properties of his serum. Physicians also look to the laboratory for conclusive results on which to base their diagnosis, and combining the fact that they have recommended this particular laboratory, with their sincere wish of keeping the good-will and confidence of the patient, they are apt to feel a decided lack of faith in the skill of the serologist, and it is this lack of faith that we wish to avoid, being at the same time sincere in our dealings with both the physician and the patient.

With the thought that it might be possible to use one of these reactions outlined in the following pages, as a corroborative test with our regular Wassermann, and also that the interest of those engaged in commercial laboratories would be aroused, and that they would be stimulated into publishing their

research work, causing a reciprocity of ideas between those so engaged, I submit this article for publication.

In many diseases, and particularly syphilis, the serum of a patient thus afflicted, when mixed with colloidal solutions of lecithin or sodium glycocholate, forms a flocculent precipitate which may be seen macroscopically. Sodium taurocholate, taurin solution and distilled water, was used by Wieder and L'Engle, while other authors have used different solutions of hydrophilic colloids with varying results. The methods mentioned in the following pages were selected, because of their simple technic, and that workers have placed them at the head of the list for researching investigation.

The first method investigated was that of Porges and Meier, who use a 1 per cent lecithin solution in normal saline, and the serum diluted 1:5 with salt solution. One c.c. of the diluted serum was mixed with 0.2 c.c. of the lecithin, incubated at 37° for one hour, and left overnight at room temperature, results being read off the following morning. Nineteen serums, eight positives and eleven negatives were used in this test, and also in the fourth method; a smaller number being used in the second and third methods. Where inactivated serum was advocated by the author, we also used activated serum, the results obtained being nearly similar. The specificity of this method is low, approximately 45 per cent of the positive serums gave negative results, and 20 per cent of the negative serums gave positive results.

The second method was that of Elias, Neubauer, Porges and Salomon. One per cent of freshly made sodium glycocholate in distilled water is used in this test. Serums must be clear and contain very little hemoglobin. Two-tenths c.c. of the serum, activated and inactivated, is mixed with 0.2 c.c. of the solution and left from 16 to 20 hours at room temperature. The results obtained were slightly better and more accurate than the first method, but the advantages of this method are greatly lessened by the fact that only clear serums can be used in the tests.

The third method tested was that of Klausner, who employs distilled water, using serum undiluted and diluted 1 to 5 with distilled water. Two-tenths c.c. of each of the serums are mixed with 0.7 c.c. of distilled water, well shaken, and kept at room temperature for 15 hours, and readings then made. Results obtained were confusing and nonspecific.

The fourth method used was that of Herman and Perutz. They employ two solutions, the first being 2 per cent sodium glycocholate in 95 per cent alcohol, plus 0.4 gram of cholesterin; and the second solution is 2 per cent sodium glycocholate in distilled water. The first solution is diluted 1:20 with distilled water before using. In the test proper one part of (a) diluted, one part of (b), and two parts of serum are mixed, shaken gently, and let stand for 18 to 24 hours at room temperature. Results obtained were distinctly better and more accurate than in any of the other methods tested, but were only partially specific. Approximately 70-75 per cent of the positives gave positive results, while two of the eleven negative serums gave distinctly positive results, and one was extremely doubtful, due to a heavy clouding. Some earlier workers found this test equally as specific as the Wassermann in syphilitic cases; however, later observers fail

to confirm these earlier researches. Olitsky and Olmstead made a detailed study of this reaction in syphilitic and nonsyphilitic serums, and found it to be 61 per cent specific in 95 syphilitics and 80 per cent specific in 80 nonsyphilitics. Summarizing our observations on the results obtained in the tests of the above methods, it is quite obvious that, possibly with the exception of the Herman and Perutz method, these methods can not be used with any great degree of certainty to corroborate the Wassermann test, and especially so in those cases where the Wassermann gives a doubtful or anticomplementary result.

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EDITORIALS

Pathology of the Pneumonia in the United States Army Camps During the Winter of 1917-18

THE Rockefeller Institute for Medical Research has issued a monograph by Professor William G. McCallum on the above-named subject. There is given a short historical survey of epidemics of pneumonia. In the sixteenth century such epidemics were well known in Italy. In 1535 one such epidemic apparently prevailed over the greater part of the Peninsula. The attack began with a chill, pain in the head and chest, and a cough at first dry but later attended by expectoration. Often the patient became delirious and later fell into a stupor. Death usually occurred on the sixth or seventh day, and the prognosis was good beyond this time. Pleural effusions were found and the lungs are stated to have been indurated and turbid, with frequent abscesses. Similar epidemics were reported in the seventeenth century. Several authors of that time reported the finding of serous and blood-stained effusions in the pleura and pericardium. For the most part, the lungs were consolidated, but occasionally rendered diffluent by suppuration. According to Hirsch so-called typhoid pneumonic epidemics were observed in England, Spain, Italy, Switzerland, France, and North America in

the eighteenth century. The type of the disease observed at that time was said to be highly malignant and the lungs were often found to contain much purulent material. In 1790 Desauvages and others described such disease among soldiers in France. They made postmortem examinations and found suppurative processes in the lungs with effusion of pus or gelatinous serum in the chest and sometimes in the pericardium. Similar epidemics were described by Noah Webster, the lexicographer, as occurring in Connecticut in 1712, 1719, 1760 and 1795. In 1812 there was a great epidemic which apparently started in Canada, spread through the northern part of the United States, and finally reached the South. There is a book by James Mann, hospital surgeon of the army, published in 1816 which gives a good description of the condition including a few postmortem observations. From these it is learned that the soldiers died with extensive pleural effusion sometimes of thick pus, as in the case of one man whose pleura was drained by an incision between the ribs, and sometimes of a thick yellowish whey-colored fluid, while a membrane of coagulated lymph lined the cavity of the thorax. Sometimes the lungs had a dark purple appearance and incision accompanied by compression forced out pus from the air cells. In other cases the lungs were contracted, compact, tough, and almost destitute of air cells. In some instances the lungs appeared like the liver, tender, easily lacerated with the fingers, and entirely destitute of the spongy texture peculiar to healthy lungs.

Even at that early date, measles as a predisposing cause of this form of pneumonia was recognized. McReynolds and Hudson described epidemic pneumonia among troops on the Niagara front. This epidemic prevailed among the soldiers in western New York in December, 1812, and January, 1813. From the soldiers and their camps it spread through New York and over the greater part of New England. The authors of that time did not describe minutely the pulmonary changes, but it is evident from their descriptions that this disease was not definite lobar pneumonia, but probably a form of bronchopneumonia with extensive pleural exudate. From 1815 to 1862 there were occasional epidemics.

Little information appears to be available regarding the prevalence of this disease among soldiers during the Civil War. Woodward gives statistical information showing that measles was prevalent, that pneumonia followed it, and that the mortality was considerable, but these conditions were overshadowed by the greater prevalence of typhoid fever and dysentery. Of the cases of bronchopneumonia following measles during the Civil War the lungs of three have been preserved in the Army Medical Museum and McCallum makes the following statement concerning his examination of one of these specimens:

"The history in Case 50 states that Private J. B. Talbot, age 23, was admitted February 13, 1864, with pleuropneumonia following measles and died February 29. Postmortem examination showed that the lower lobe of the right lung was hepatized, red, and presented on section a number of small superficial abscesses. The pleura was thickened. The lung is fibrinous, exudated, and most of it is spongy in texture, but it contains some distinct, firm nodules which are branched at times."

McCallum found that sections of this lung no longer stain well, but the outlines of cells may be discerned. There is in the section a patch of consolida-

tion. There are central bronchioles which contain exudate and have lost their epithelium. The walls are thick and infiltrated with mononuclear cells and are penetrated by many distended blood vessels. The adjacent alveolar walls are infiltrated and contain dense plugs of fibrin with desquamated epithelium. The larger area of consolidation shows several bronchi with the same change. The walls of a rather large one are especially infiltrated. More peripheral alveoli have dense contents of fibrin and epithelium. In the more central part of the patch the tissue is disintegrated and full of small broken cells, possibly leucocytes. There is a mass of granular material at one spot resembling bacteria. With proper stain, McCallum was able to demonstrate streptococci in the midst of the necrotic area. No other bacteria could be seen. There is no doubt of the existence and rather wide prevalence of streptococcal pneumonia among Northern Soldiers during the Civil War. Since that time other epidemics of pneumonia have been described, but it is difficult from the clinical description to determine the nature of the disease.

This form of bronchopneumonia was most carefully and skilfully investigated by Delafield. His understanding of the contrast between this type of pulmonary affection and the sort of exudative process which follows the aspiration of infected materials into the bronchi, or the other examples of terminal patchy pneumonia characterized by the filling of the alveoli which are connected with the infected bronchioles with an exudate of polymorphonuclear leucocytes, is satisfactory and his descriptions form a model of conciseness.

During the present war epidemic pneumonia has been carefully studied by many English physicians. They describe a purulent bronchitis which is fatal and from which they isolated the influenza bacillus in the sputum in most cases. However, they found other organisms including the pneumococcus, streptococcus, and staphylococcus.

Coming to his own work McCallum thinks that emphasis should be laid upon the importance of measles in the inception of this epidemic. It is well known that measles prevailed during the winter of 1917-18 most largely in camps filled by southern and rural troops, and it was in these camps that pneumonia most frequently followed measles. It should not be understood that bronchopneumonia did not occur except among those who had recently had measles. It did occur among both the measles and the nonmeasles group, but it was more common and much more deadly among the measles group. McCallum is of the opinion that soldiers who had recently had measles and therefore were in a condition of reduced resistance fell the first victims to the streptococcus. This organism having grown lusty and increased in virulence, found itself able to overcome the resistance of the most virile and was no longer compelled to await the debilitating effects of measles. We quote the following:

"The influenza bacillus was frequently in the sputum and occasionally in the lungs and pleural cavity. It was always associated with the streptococcus and it is difficult to assign it a predominant part in the causation of the pneumonia. In general, the pneumococcus of whatever type produced lobar pneumonia and the streptococcus either what we shall describe as interstitial bronchopneumonia, or another lesion to which for convenience we assign the name lob-

ular pneumonia. The exact biological nature of this streptococcus and its identity in different camps are still subjects which are actively discussed. It seems to be the same wherever it has been found, but it will require time to determine whether there are several strains at work. Indeed from many camps there come reports of numerous cases due to a nonhemolytic streptococcus. All these important points must be determined by further study which must also decide the significance of the influenza bacillus, staphylococcus, and other organisms in the production of the disease. Our own experience has led us to believe that a fairly constant hemolytic streptococcus is the important factor."

McCallum's studies were carried on principally at Fort Sam Houston, Texas, and at Camp Dodge, Iowa. Details are given concerning 44 cases studied in Texas and 40 in Iowa. The monograph contains well executed plates giving visual representation of the findings. Lobar pneumonia caused by the several types of pneumococcus was found, but the number of cases seems not especially different from what ordinarily occurs during corresponding months of the year. In some cases lobar pneumonia was combined with streptococcal pneumonia; in others the findings were in no way dissimilar to those usually observed in straight pneumococcal infections. The streptococcal infection seems to occur by way of the upper respiratory tract and the illness begins with a sore throat, cough, and fever. Physical signs in the lungs are indefinite until evidence of accumulation of fluid in the chest appears. Dyspnea of extreme type and especially during inspiration is characteristic and is indicated by a marked cyanosis. Most patients are alert, apprehensive, and sleepless, and there is often delirium. The first type of pulmonary lesion described is designated as interstitial bronchopneumonia. It is the result of infection of the bronchioles with hemolytic streptococci leading to marked changes in the bronchial walls and adjacent pulmonary tissue. The most striking accompaniment of this is collapse of the lung, affecting those portions formerly supplied with air by the obstructed bronchi, together with the formation of nodules of consolidation about the terminal portions of the bronchi. At the same time there is extreme engorgement of the blood vessels and hemorrhage into the tissue about the nodules of consolidation. The walls of the bronchi become densely infiltrated with mononuclear cells. Extreme hyperemia and the new formation of blood vessels and of connective tissue add greatly to the thickening and deformity of the walls. In some cases the whole lining of the bronchi becomes necrotic and presents the appearance of a diphtheritic pseudomembrane. Coincident with the changes in the bronchial walls are similar alterations in the walls of the adjacent alveoli. The alveolar capillaries are distended with blood, the epithelium is proliferated rapidly and desquamated into the alveolar cavity. These cavities are found to be filled with blood, fibrin, and desquamated epithelial cells. Those more distant contain smaller numbers of these cells and are filled with a thick viscid fluid. This gelatinous edema gives a semblance of confluence between the areas of consolidation before it is really completed. There are many cases in which the changes are restricted to the neighborhood of the bronchioles and separated from each other by wide areas of air-containing tissue. Streptococci are found in masses in the lumen of each bronchus or bronchiole mingled with the leucocytes of the

exudate, but in most instances they are not found abundantly in the substance of the tissue or in the alveolar contents.

The lymphatics which run from the pleural network by way of the interlobular septa, bronchial walls, and blood vessels to the nodes at the hilum of the lung quickly become a channel for the transport of these bacteria. Great numbers of bacteria are found in these canals and in the sinuses of the lymph nodes at the hilum where they appear to be detained. They cause thrombosis of the lymph channels which become distended to an enormous size and appear to the unaided eye as most conspicuous, beaded, white strands on the cut surface of the lung extending to connect with the pleural network, which may be in places similarly injected and distended with a white, infected, semipurulent thrombus. It is thought that bacteria extend by growth along these obstructed channels in both directions, and thus passing from the lung to the pleura, infect the walls of the pleural cavity and set up intense inflammation resulting in the formation of fluid exudate.

From the beginning of the infection of the pleura the effusion of exudate becomes a dominant feature both in clinical signs and anatomical changes. It proceeds with great rapidity and compresses the lung to such a degree as to complete the atelectasis. When the exudate accumulates in large quantities, such as two or more liters, the lung becomes an airless, pasty, blue clump of tissue which is plastic against the pericardium or back against the side of the vertebral column. Later the pleural exudate becomes more purulent, having begun as a thin, greenish, turbid fluid filled with streptococci, disintegrated leucocytes, and shreds of fibrin.

Rapid organization of the inflammatory exudate is everywhere in evidence. This affects the alveolar and bronchial contents which are replaced in part by connective tissue resulting in interstitial new growth which thickens the alveolar walls, the bronchial walls, the adventitia or blood vessels, and the interlobular septa. The thrombosed lymphatics become organized and occluded permanently by fibrous tissue so that new ones must eventually be formed, while absorption in the meantime is greatly hampered. Most striking is the rapid organization of the layer of fibrin which covers the pleura. This results in the formation of thick vascular granulation tissue on the pleural surface, adhesions between contiguous surfaces are rendered permanent, and in the later stages incapsulated pockets of intrapleural origin filled with pus are not infrequently formed and offer difficulties to the surgeon.

Less frequently large portions of the lung become necrotic. Such material is loaded with streptococci and is often discharged through the bronchus, leaving a cavity. When such a cavity communicates with the pleura, pneumothorax may result, or if the chest is already open it appears as a bronchopleural fistula. Such a disease of the lung betrays its tendency to heal. Everything except perhaps the bronchial exudate, the pleural exudate, and the intrapulmonary necroses or abscesses partakes of the nature of a barricading process. All changes tend to limit the advance of the streptococci and to this end the tissue is indurated. The alveolar exudate with its fibrin later replaced by connective tissue, the thrombosis of the lymphatics, the thickening and granulation tissue formation in the

bronchial walls, interlobular septa, and pleura, and the wide-spread hyperplasia of lymphoid tissue throughout the lung, are all evidence of a considerable power of resistance which permits the body time for defense. The infection is fairly well localized in the respiratory tract and there is no septicemia until perhaps the last few hours of life. Other organs suffer but little.

The type designated as lobular pneumonia is shown in cases in which the streptococci invade in the same way. One does not know how to distinguish the symptoms and yet at autopsy although sometimes even the lungs may look much the same at a casual glance, they are really different. There are no firm peribronchial nodules. There are patches of consolidation in which the alveoli are filled with blood, leucocytes, and enormous quantities of streptococci. There are no interstitial changes and no signs of resistance. Widespread necrosis occurs with final destruction of areas of lung tissue. Hemorrhage about these areas is usual and there is often pleural effusion without any organization of the exudate. There are cases in which this lobular pneumonia occurs in pure form, but there are many more in which it is found side by side with the interstitial bronchopneumonia. There are different expressions of reaction to the same infection, but in these the reaction is feeble. There is nothing new in the forms of pneumonia in this epidemic. They have existed always and, historically, can be traced back at least to the sixteenth century. More scientific descriptions date from 1812, and perfectly clear ones from Delafield in 1884.

—*V. C. V.*

---

*Shock**

ONE of the most striking developments of modern pathology—not to say physiology—is that which relegates the nervous system to a distinctly subordinate place. This development has resulted in placing chemical and physicochemical action in a commanding position, and in assigning to the nervous system a sort of controlling function. In the absence of a nervous system pathologic changes develop, as they do in the presence, but the rate of development is abnormally slow. Evidently the nervous system influences the speed of reactions. Nowhere is this more striking than in shock.

It has not been so long since shock was referred to nervous impulse alone. Extreme nervous irritations acting, over a sufficient period of time, upon the vasomotor centers lead to fatigue of those centers, with the result that the vessels—chiefly the arterioles— lost their tone and power of constriction, and that the blood pressure fell, and was recovered only with the greatest difficulty. Later observations showed that the reverse was true; i. e., that the vasomotor mechanism remained intact; that instead of a dilatation of the arterioles, they were constricted. Despite this arterial state the blood pressure fell, and remained low during the progress of the state of shock.

*Traumatic Toxaemia as a Factor in Shock. Reports of the Special Investigation Committee on Surgical Shock and Allied Conditions. Special Rept. No. 26. Medical Research Committee. Oxford University Press, March 14, 1919.

Later also it was found that the low blood pressure was the result of lack of blood volume. When hemorrhage had occurred this was readily understood; when it had not occurred it was explained with difficulty. Blood examinations in the latter type of cases showed that the blood was concentrated; i.e, that the corpuscles were increased in unit volumes, and that the plasma was decreased. Evidently therefore the loss of blood volume was due to loss of fluid from the blood, but not to loss of the formed elements, and, since hemorrhage had not occurred, the fluid must have passed into the tissues. So the problem was shifted from the vessels to the tissues. Why did the fluid of the blood pass outward? Why should the fluids be attracted to the tissues? It is one of the great contributions of Y. Henderson, that he focused attention upon this aspect of the problem. Henderson believed that an early symptom of shock was hyperpnea, caused by the sensory impulses originating in a damaged area of the body, and that this resulted in a washing out of CO_2 from the lungs and vascular system. So a condition of acapnia was produced which acted to reduce the diastolic pressure in the heart by reducing pressure in the veins. At the same time the ratio of CO_2 in the blood to CO_2 in the tissues fell and this led to a flow of water from the blood to the more acid tissues. In other words a condition of more or less generalized tissue acidosis occurred which led to edema of the various organs, and coincident concentration of the blood.

At the beginning of the war these two conceptions of shock were held; the one which laid the failure of the circulation at the door of vasomotor fatigue—the result of overstimulation of the centers—and the other which held that the vasomotor centers were abnormally active, and that the main factor was a condition of tissue acidosis which resulted in concentration of the blood to such an extent that the heart no longer was supplied with enough blood to pump, even when the arterioles were in a state of maximal constriction. The war experiences have tended to discredit the former theory and to support the latter.

The observations at the front in Casualty Clearing Stations and First Aid Stations have been made the basis of experimentation. One prime conclusion has been reached; i.e., that muscle injury can produce a state of shock, and that absorption of toxins or similar substances can do the same. These conclusions rest upon the following clinical evidence: (1) The experience that, putting aside gross injuries, the shock from which the abdominally wounded man suffers, depends more on the loss of blood, or on peritonitis and sepsis, than on the extent of the lesion. Many men thought by the surgeon to have died of shock, have been proved by postmortem examination to have died of acute sepsis or gas gangrene. (2) The striking change for the better that follows excision of muscles infected by anaerobic bacilli. (3) The well-known association between shock and small multiple or single large muscle wounds. (4) The shocking nature of operations involving large muscle masses (amputation at the hip). This clinical evidence may be summarized by saying that shock (secondary shock) is the result of an intoxication. What are the toxins?

In certain cases it may be that bacteria are the active causes of shock; that they produce substances which bring about the whole sequence of events. Such a state of affairs we might call bacteriotoxemic shock. In certain other cases in which the bacteria are not present, there must be other substances, not of bac-

terial origin, but of tissue origin which cause the typical sequence of events. This we might call histo-toxemic shock. Recent experimental work has concerned itself to a large extent with the latter, and with such evidently successful results that we may well wonder whether the former is not the result of bacterial action upon the tissues, setting free toxic substances,—in other words, whether after all the condition of shock is not always a tissue affair, hastened or modified in certain cases, by bacteria.

At the front, shock was associated with other conditions than muscle damage and infection; i.e., hemorrhage, pain, cold, mental distress, and rough handling. Elimination of cold and rough handling were most effective in treatment. Warmth tended to improve the circulation; gentle treatment tended to reduce muscle damage and pain. Administration of morphine in small doses was valuable because it relieved pain, and tended to quiet. Large doses of this drug were dangerous, probably because they tended to decrease oxidations in the body. Administrations of fluid were of very real value, because of the replenishment of the water in the blood vessels. Gum (colloidal) solutions given intravenously were valuable because the gum tended to retain the absorbed water and keep it in the vessels, and so reestablish the blood volume and pressure.

Wallace says that acidosis can be neglected as a cause of shock, but it would seem that the whole sequence of the events, and the methods of treatment point to the fact that tissue acidosis is the essential condition of the complex, and that to it are due the various clinical manifestations. Whether hyperpnea with subsequent acapnia, or a primary toxemia due to tissue destruction, or loss of blood alone forms the starting point of the complex, any one or all combined tend to produce a chemical state in the organs and tissues of the body which may logically be called acidosis, which is the condition upon which depends withdrawal from the blood vessels. The effects of anesthetics point to the same conclusion, for chloroform is the most dangerous one, ether follows it, and gas-oxygen is safest.

Cannon, Bayliss and others have subjected animals to experiments to determine the actual condition supervening upon muscle damage. They found that if they ruptured and severely bruised muscles, without breaking the skin, the muscles underwent considerable swelling and some extravasation of blood though not sufficient to produce symptoms of hemorrhage. During the process of traumatization the blood pressure fell and was followed sometimes by a temporary rise, after which a secondary fall occurred. The promptness of the primary fall suggested that it was reflex, but it occurred after the cord had been cut. It failed to appear if the aorta was closed above the iliacs during the traumatization although the nerve conduction would not be impaired during this short anemia. There was no change in the alkaline reserve during the period of the primary fall. The observations indicate that the sharp fall is not due to any general effect of the trauma on the circulation brought about through nervous agencies or liberated acid. It may be due to a combination of dilatation of blood vessels in the injured area together with some extravasation of blood, with the rapid setting free of a chemical substance acting as a powerful depressant on the blood pressure. The secondary fall may be explained by the continued absorption of the depressant substance and by the establishment of a vicious circle in consequence

of the low blood pressure. This is associated with a steady fall in the bicarbonate reserve of the blood. A remarkable effect on the respiration is usually to be seen. The rate increases greatly and the depth is shallow. These experimental results are strikingly similar to cases of shell traumata seen in men wounded in battle. In all such cases the shock was accompanied by acidosis.

Erlanger and his associates have observed that temporary exclusion of the circulation from any considerable part of the body results in shock. Cannon and Bayliss have made similar observations and they point out that muscles produce certain products, especially when the oxygen supply is diminished or cut off, which, when set free in the circulation, lower the blood pressure. At the same time the alkaline reserve falls. They also have shown that massage of damaged muscles, results in a further fall of blood pressure.

Bayliss says it appears that the conclusion must be drawn that the toxic products can be removed or destroyed as quickly as they pass into the blood current if the volume of the blood is kept sufficiently large to insure a good circulation and supply of oxygen to the tissues. Whether the toxic products are oxidized, as lactic acid is, or rendered harmless by passing through the liver, is not known as yet. We know that histamine disappears very rapidly if the circulation is effective.

A large amount of recent experimental work has concerned itself with this substance histamine which is a protein split-product, a derivative of the amino acid histidine. Injection of this substance into the veins of experimental animals produces a condition that is typically shock. The blood pressure falls and the respirations become feeble. At first sight one receives the impression that the heartbeat has become feeble almost to extinction; but if the chest is opened, the heart is seen to be beating strongly and regularly, but its chambers contain remarkably little blood. The veins and arteries seem to contain but little blood and are constricted. The liver is pale and certainly not distended with blood, and the same is true of the spleen. The blood itself is markedly concentrated. It is an interesting fact in this conclusion that animals can tolerate only exceedingly small doses of histamine if this operation is combined with ether anesthesia.

The whole trend of the work up to the present time seems to indicate in a very definite way that secondary shock is the expression of a toxemia which is frequently the result of tissue damage. Such damage sets free, of itself, or with the assistance of bacteria, substances like histamine which are products of incomplete protein splitting, which, in the presence of sufficient oxygen supplies, are readily removed. Any circumstances that decrease oxidation accumulate the shock.

—P. G. W.

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[1]Billings, J. S.: Our Medical Literature, Trans. VII Intern. Med. Congress, Lond., 1881, i, 54-70.

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[4]Place, F.: Bibliographic Style in Medical Literature, Med. Record, N. Y., 1913, lxxxiii, 157-160.

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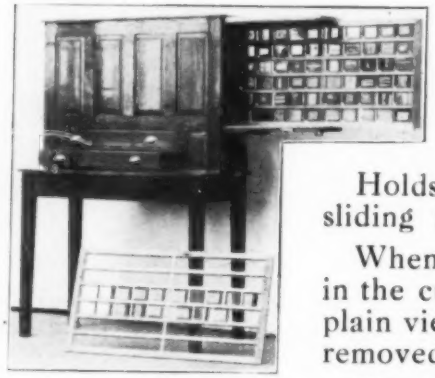

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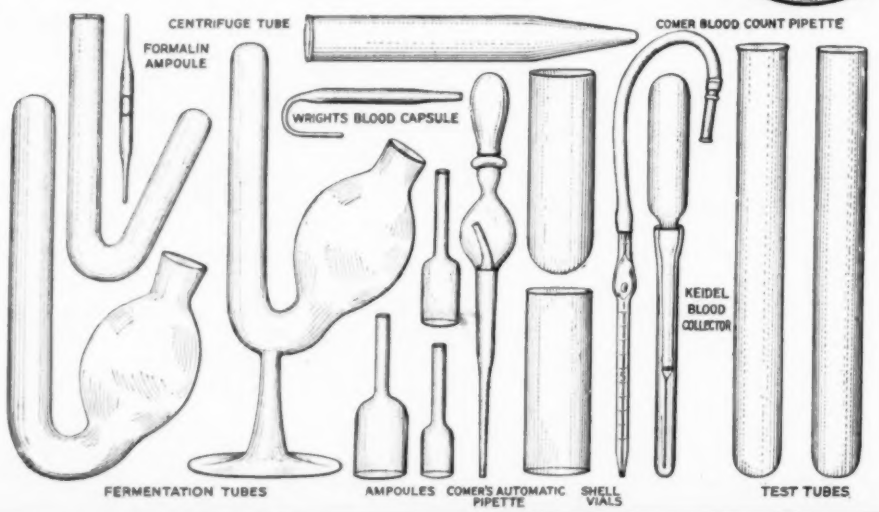

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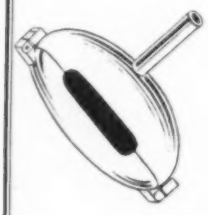

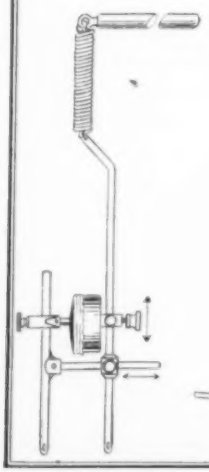

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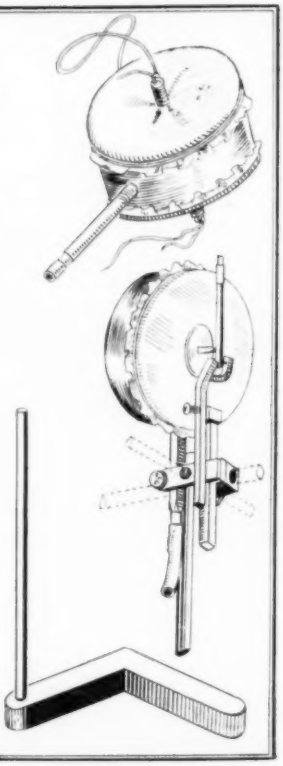

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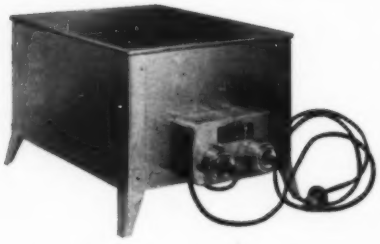

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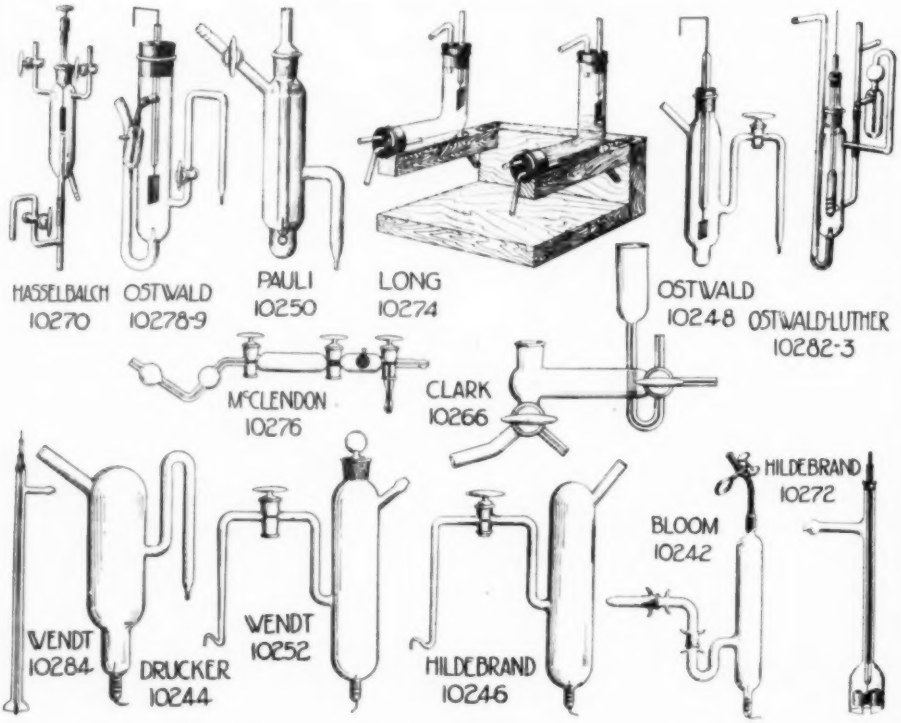

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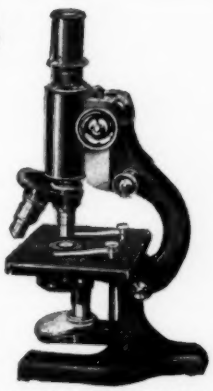

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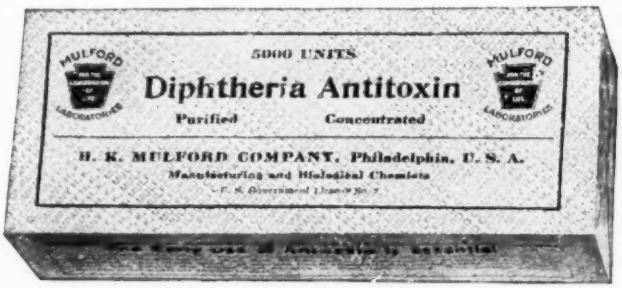

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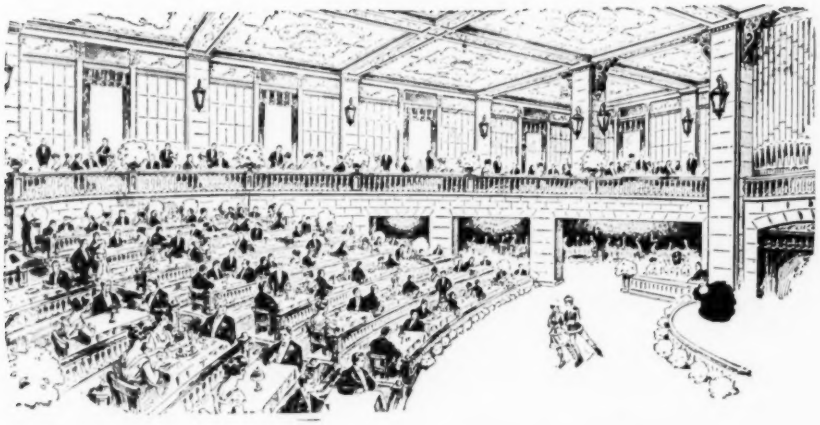

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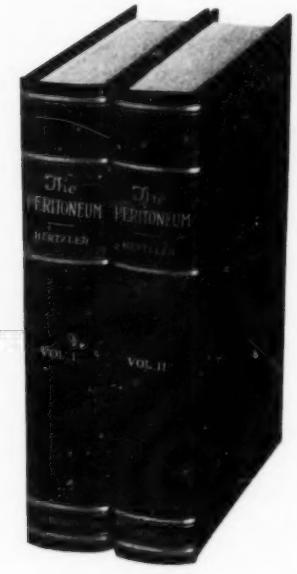

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Our Exhibit at the National Chemical Exposition, September 22-29, will be in the Coliseum Building, Booths 214-215.

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